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Clemson Univ, SC Dept of Mechanical Engineering

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Evaluation of Hyperfiltration for Separation of Toxic Substances in Textile Process Water

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16. ABSTRACT The report gives results of an evaluation of hyperfiltration for separation of toxic substances in textile process water. Three membranes (cellulose acetate, polyether/amide, and dynamic zirconium oxide/polyacrylic acid) were used to separate process water from scour and dye operations into permeate and concentrated streams. Feed, permeate, and concentrate samples from each run were analyzed. Chemical analyses for organic and metal toxic pollutants and bioassays for rat acute toxicity, fathead minnow and daphnia acute toxicity, microbial mutagenicity, and hamster ovary clone cytotoxicity response were conducted. The minnow and daphnia tests showed active results, with good correlation. The other bioassays produced no response. Toxicant rejections of 55 to 100% were observed: the relative rejection by the three membranes was almost exclusively counter to the relative rejection of salt. Mass balances of biological toxicant were excellent, suggesting high confidence in the result. Chemical analysis for organic compounds sensed 19 of the organic toxic pollutants in low levels, <300 mg/cu m. The results were difficult to interpret for mass balance and membrane rejection of particular solutes. Except for a few compounds, the data appear to suggest membrane separation. Metal toxic pollutant concentrations were low: only three were concentrated enough for valid estimations.

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Toxicity	Scouring	Hyperfiltration	06T	13H
Textile Industry	Dyeing	Process Water	11E	
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ABSTRACT

Three hyperfiltration membranes (cellulose acetate, poly ether/amide, and dynamic zirconium oxide/polyacrylic acid) were used to separate textile process water from scour and dye operations into permeate and concentrated streams. Samples of feed, permeate, and concentrate from each run were obtained and analyzed. Chemical analysis for organic and metal toxic pollutants and bioassays for rat acute toxicity, fathead minnows and Daphnia acute toxicity, microbial mutagenicity, and hamster ovary clone cytotoxicity response were conducted.

Both the fathead minnows and Daphnia tests showed results in the active range. The other bioassays did not. The results were consistent in indicating a substantial reduction of toxicant in permeate samples from all membranes and corresponding increases in toxicant in the residual concentrate samples. Toxicant rejections of 55 to 100 percent were observed, and the relative rejection by the three membranes was almost exclusively counter to the relative rejection of salt. Mass balances of biological toxicant were excellent, suggesting high confidence in the result.

Chemical analysis for organic compounds sensed 19 of the organic toxic pollunts in low levels (300 mg/m^3 and under). The results were difficult to interpret for mass balance and membrane rejection of particular solutes. Except for a few compounds, the data appears to suggest membrane separation. An experiment set devised to enhance accuracy of analysis is recommended to establish the rejections of pertinent substances.

Metal toxic pollutant concentrations were low. Analysis revealed only three in high enough concentrations for reliable estimation of performance. Other metals analyzed and the toxic metals results agree with the historically high rejection of metals (reference page 21).

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SYMBOLS AND UNITS

Item	Symbol	(Unit)
Pressure	P	(N/m^2)
Temperature	T	(°C)
Recovery	R	(no units)
Concentration	C	(g/m^3)

Subscripts

Feed - f
Permeate - p
Concentrate - c

Units (S.I.)	Multiply By	To Get Unit
m	3.28	ft
°C (°K-273.16)	1.8	°F-32
MN/m^2	$1.44 \times 10^{+2}$	psi
m^3	264	gallon
m^2	10.76	ft^2
S (Siemens)	1.00	ohm^{-1} (mho)

ℓ (liter) is used generally rather than the S. I. unit dm^3

Metric Prefixes

M denotes 10^6
k denotes 10^3
m denotes 10^{-3}
μ denotes 10^{-6}

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INTRODUCTION

The U. S. Environmental Protection Agency (EPA) is implementing limits on industrial plant discharge of Consent Decree Toxic Pollutants and developing technologies for compliance with these limits. The textile industry discharges large quantities of effluents with some effluents containing detectable concentrations of several toxic pollutants.[1] Other chemicals not included in the Consent Decree such as dyes which are toxic at concentrations as low as 100 g/m^3 may be present in the typical discharge.[2] This report describes an investigation of hyperfiltration as a technology for separating toxic materials occurring in selected textile process effluents.

The purposes of the investigation were: 1) to determine the effectiveness of representative commercial hyperfiltration membranes in separating toxicity, as measured by EPA-approved short-term bioassay, found in the untreated process effluents; 2) to compare the toxic rejections of the membranes; 3) to obtain rejection coefficients of the detectable solutes; and 4) to correlate toxicity with the presence of detectable solutes, evaluating internal consistencies among both the bioassay results and the chemical analysis results.

It was desired to obtain representative samples of untreated process effluent and process them by hyperfiltration. Samples of feed, permeate, and concentrate could be analyzed for specific chemicals and be subjected to bioassay. The fluids selected were of a cotton scour and a cotton dye process from a dye range.

Membranes selected have a reasonable expectation of industrial applicability. Those selected were commercial cellulose acetate, poly(ether/amide), and a dynamic membrane (zirconium oxide/poly acrylic acid) prepared at Clemson University. The polyamides were eliminated due to expected difficulties with plugging from the industrial fluid, and other membranes were not considered to be sufficiently commercial at the decision time.

A test program was designed for the fluids and membrane combinations cited. The samples were analyzed by Monsanto Research Corporation or designa ed subcontractor under separate contract to EPA. Analyses selected were organic toxic and metal toxic chemical analysis; rat acute toxicity; Fathead minnow 96-hour acute toxicity; Daphnia 48-hour acute toxicity; microbial mutagenicity response; and hamster ovary clone cytotoxicity. In addition, measurements of total solids, electric conductivity, pH, absorbance (410 nm), and infrared spectra were performed at Clemson University.

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[1] Rawlings, G.D. and Max Samfield," Source Assessment: Textile Plant Wastewater Toxics Study Phase I," EPA 600/2-78-004h, March, 1978.

[2] "Dyes and the Environment," ADMI Report, Volume II, September, 1974.

CONCLUSIONS

1. Hyperfiltration membranes have been shown to be effective in producing a substantially less toxic (to aquatic organisms) permeate while also producing a correspondingly more toxic concentrate when operated on actual textile plant effluents.

2. While all membranes tested were effective, the relative separation of toxicants was observed to be counter to the relative salt separation. That is, the membrane having the best salt rejection was not the best with regard to toxic material rejection.

3. The membranes exhibited high rejection, greater than 0.85, of solute components detected by color, total solids, and conductivity analyses.

4. All the metal toxic pollutants were detected, but only three were present in concentrations sufficient to calculate reliable rejection coefficients. These were high, the average values were: above 0.89 for arsenic, 0.97 for copper, and 1.00 for zinc. This result coupled with prior experience of generally high rejection of metal ions found in textile process effluents provides good evidence for high rejection of toxic pollutant metals in these effluents.

5. Only 19 organic toxic pollutants were detected, also at low concentrations. Because of the analytical difficulties associated with low concentration and difficulty in controlling concentrations of volatile organic solutes at elevated temperatures during the experiments reliable rejection coefficients were not obtained for the organic toxic pollutants. However, using decreased solute concentration in the permeate and/or increased solute concentration in the concentrate as indication of rejection, most solutes were rejected in these process effluents, i.e., 43 of 51 comparisons showed positive rejection.

6. Because so few rejection coefficients were evaluated no cause/effect correlations between toxic response and specific toxic pollutants were apparent. Correlations between aquatic organism toxicity and concentrations of copper and arsenic appear strong. However, the metal concentrations were likely too low to account for the toxicity.

7. Toxicant concentrations implied by the aquatic organism toxicity assays permitted calculation of reasonable toxicant mass balances. The toxicant concentrations were substantially proportional to the total solids concentrations.

8. It should be noted the correlation coefficient relating the toxicant concentrations implied by the two aquatic organisms Fathead minnows and Daphnia was high, 0.94, suggesting that for these two discharge streams, a measurement of either individual assay would have produced parrallel data.

9. Rat toxicity and bacterial mutagenicity tests produced no response. Concentrates were cytotoxic, but no cytotoxicity was observed in feeds and permeates. Cytotoxicants were probably concentrated (rejected).

RECOMMENDATIONS

1. The observed significant separation of toxicity provides a basis for recommending hyperfiltration be considered further as a technology for toxic control of industrial effluents.

2. Continued research to quantify the applicability of this technology is recommended. Specifically, the analytical and concentration control difficulties experienced in this field experiment suggest well controlled, repeatable, zero recovery laboratory experiments using a few selected solutes to determine accurate rejection coefficients. The solutes should be selected to provide a breadth of properties sufficient to test models for the prediction of rejections of all the toxic pollutants. In addition, experiments using process effluents spiked with known quantities of selected solutes should be completed to permit the quantitative analysis of membrane performance under conditions approaching the field experiments conducted in this investigation.

3. Research to identify the process effluent components responsible for the toxicity to aquatic organisms is recommended.

RESULTS AND DISCUSSION

FLOWS, VOLUMES, and PHYSICAL PARAMETERS

Experiments were carried out using three hyperfiltration membranes, poly(ether/amide) composite (PEA), asymmetric cellulose acetate (CA), and zirconium oxide/poly(acrylic acid) dynamic membrane (DM), using two types of process effluents, cotton scour and dye wash. The permeate flow rates of the three membranes during the course of the experiments are presented in Figures 1 and 2.

Total solids, electric conductivity, absorbance, and pH of the samples are shown in Table 1. The general level of solids shows the effect of membrane separations and is in agreement with the concentrate levels as well. Infrared spectra obtained from sample residuals are included in Appendix A.

TABLE 1. Experiment Results for pH, Solids and Conductivity

Sample Number	Description	pH	Conductivity (μs/cm)	Total Solids (g/m^3)	Absorbance (410 nm)
1	Plant	6.6	106	15	0.
2	Apparatus	7.2	157	43	0.055
Run #3					
3	Sc-1, feed	9.7	710	730	0.050
4	Sc-1, permeate, PEA	7.2	25	105	0.
5	Sc-1, permeate CA	7.7	24	32	0.
6	Sc-1, concentrate	9.8	3830	6020	0.050
Run #4					
7	Sc-2, feed	10.4	957	870	0.03
8	Sc-2, permeate, DM	9.3	280	205	0.01
9	Sc-2, concentrate, DM	9.4	2870	3840	0.15
Run #1					
10	Dye-1, feed	6.5	271 (228)[a]	462 (391)[a]	0.1 (0.08)[a]
11	Dye-1, peameate, PEA	6.9	20	15	0.
12	Dye-1, permeate, CA	6.7	22	45	0.
13	Dye-1, concentrate	7.6	1800	2670	0.65
Run #2					
14	Dye-2, feed	7.5	929	760	2.0
15	Dye-2, permeate, DM	8.2	106	60	0.
16	Dye-2, concentrate	8.4	3230	2160	7.8

[a] In Run 1, the feed was concentrated by an estimated 18 percent before the feed sample was obtained. The estimated actual feed conductivity, solids, and absorbance values are respectively shown in parentheses.

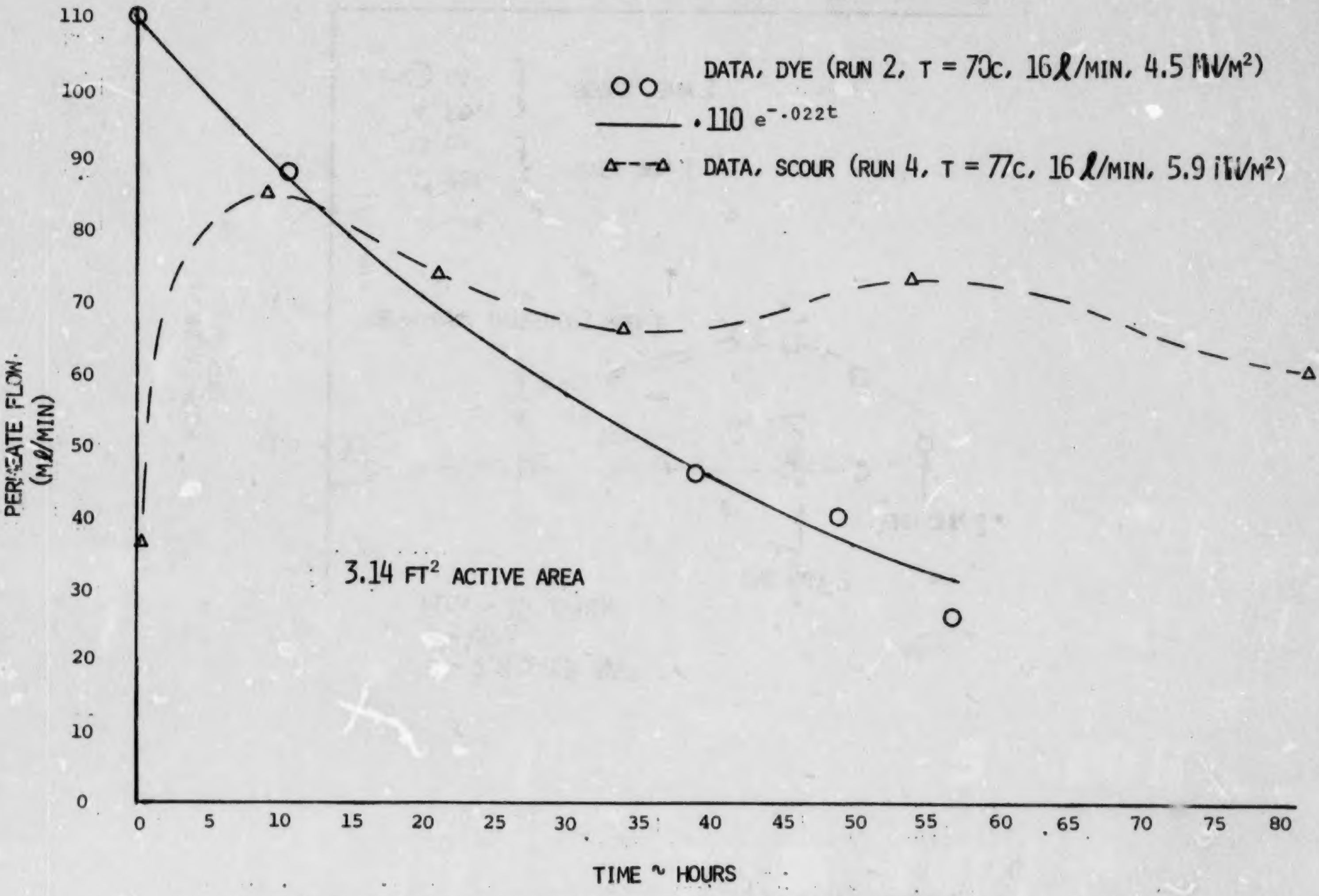

FIGURE 1 PERMEATE FLOW FROM DYNAMIC MEMBRANE ON DYE WASTE AND SCOUR WASTE

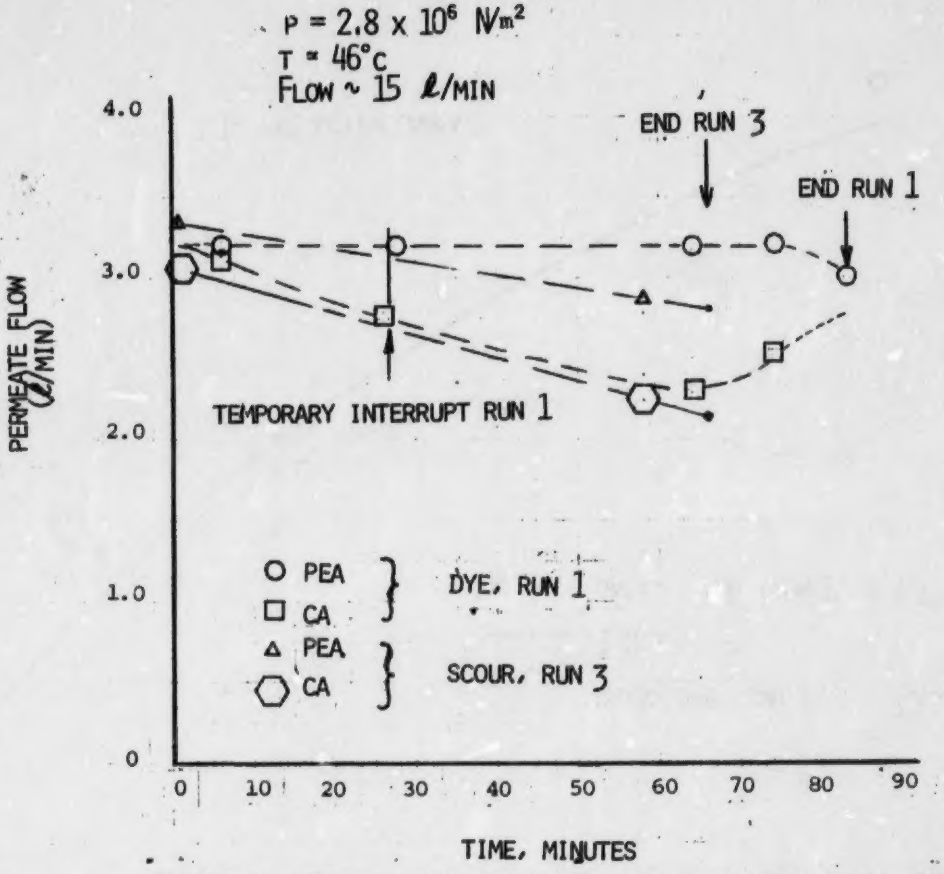

FIGURE 2 PERMEATE FLOW RATES FOR CAST MEMBRANES DURING TESTING

The volumes of permeate, feed, and concentrate have been refined as described in Appendix B., based on the total solids measurements. In general, the refined volumes agree well with direct observations forming a reasonable consensus. The volumes observed have been modified to the recovery (volume of permeate/volume of feed) shown in Table 2. These recoveries indicate the best combined agreement with final (solute) mass to initial mass ratio, rejection performance indicated by total solids analysis, and original volume estimates. The recovery ranges from 0.73 to 0.89 for the four tests, averaging 0.83. An overall mass ratio of total solids as shown in Table 2 is excellent except in run 2 where 26 percent of the original mass is not accounted for.

TABLE 2. Total Solids Balance and Recovery Data

Run	1	2	3	4
Fluid	Dye	Dye	Scour	Scour
Membrane	Cast	Dynamic	Cast	Dynamic
Recovery[a]				
Overall	0.863	0.730	0.890	0.820
Cellulose acetate	0.379	-	0.418	-
Poly(ether/amide)	0.484	-	0.472	-
Mass ratio[b] (final/initial)	0.99	0.74	0.99	0.99

[a] See Appendix B for details of the calculation of recovery.

[b] To calculate mass ration, use solids data from Table 1.

mass in PEA permeate = 0.484 x 15 = 7.26
mass in CA permeate = 0.379 x 45 = 17.05
mass in Concentrate = (1 - .484 - .379) x 2670) = 365.8
Total, mass at end of run = 390.1 g/m^3 of feed
Mass in feed = 1 x 391 = 391 g

$$\text{Mass ratio} = \frac{390}{391} = 0.99$$

In Run 2, a leak of 7 percent of feed during the run must be accounted for, depressing the mass at the final condition.

An effort to refine the calculation of rejection to include individual toxic components was made but was considered not appropriate for the analytical results obtained. The accuracy estimates given by Monsanto Research Corporation are ±100 percent for organics and ±20 percent for the metal analysis. The calculated rejections are, therefore, not highly accurate estimates. A simple, yet reasonably accurate, estimate of rejection based on permeate and feed concentrations was used. It can be shown that such a calculation is only mildly dependent on the recovery and therefore a single relation of rejection versus permeate to feed concentration ratio was used for simplicity.

Figure 3 shows the proposed relation between rejection and permeate to feed concentration ratio. It is based on a simple assumption of uniform rejection, independent of concentration, and a volume recovery of 0.85. The

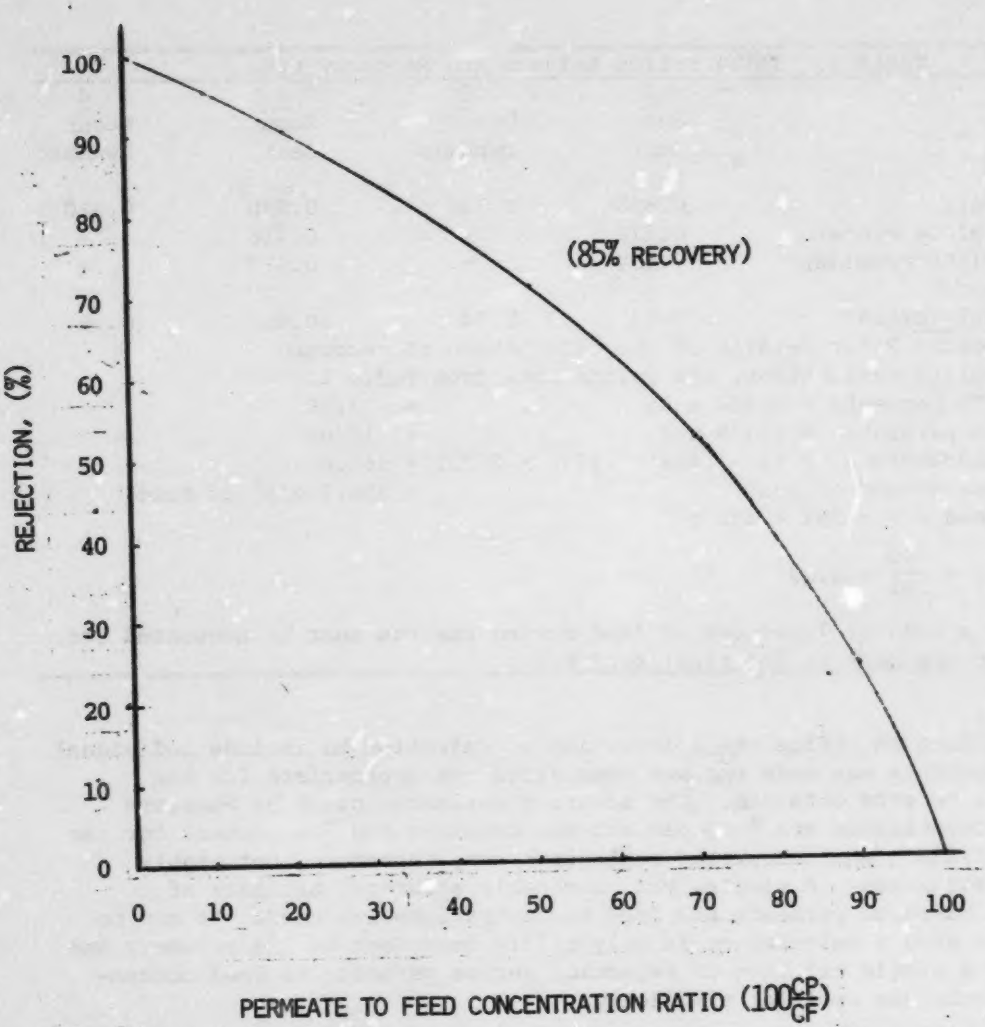

FIGURE 3 RELATION BETWEEN FEED AND PERMEATE CONCENTRATION AND MEMBRANE REJECTION

effects of vapor loss, small leaks, and recoveries different from 0.85 are estimated to be relatively minor. The use of Figure 3, or equivalent, is used to obtain rejection from permeate and feed analysis data.

The data presented in Table 1 has been analyzed for rejection and presented in Table 3. All membranes are effective in rejecting total solids and ionic solutes. The lower rejection of the solutes in scour by the dynamic membrane is probably due to its passage of ions at the $p^H \gtrsim 10$ operating condition in this fluid. All membranes were effective in removing color as evidenced by the absorbances in Table 1. The cellulose acetate permeate did not foam, while the others did produce some foam.

TABLE 3. Rejection by Membranes

Membrane/Fluid	Run Number	Rejection based on	
		Solids	Conductivity
Cellulose acetate/dye	1	0.94	0.95
Cellulose acetate/scour	3	0.98	0.99
Poly(ether/amide)/dye	1	0.98	0.96
Poly(ether/amide)/scour	3	0.93	0.99
Dynamic/Dye	2	0.97	0.95
Dynamic/Scour	4	0.88	0.85

Organic Solutes

Chemical and bioassay tests were conducted under separate contract to Monsanta Research Corporation (MRC). The complete test results as obtained from MRC are appended to this report as Appendix C for convenience. The data obtained thusly are described in detail in the following.

Tables 4, 5, 6, and 7 show the results obtained for toxic organic solutes in the four runs. The concentrations of the feed sample, permeate sample(s), and concentrate samples are shown followed by the mass ratio calculated thereform. The calculation of mass ratio is illustrated by the following example in Run 1, Bis(3-ethylhexyl) phthalate (see Table 4).

Volume data from Table 1
Concentration data from Table 4
mass in PEA permeate = 0.484 x 31 = 15.0
mass in CA permeate = 0.379 x 3 = 1.1
mass in concentrate = 0.137 x 51 = 7.0
end of run, total = 23.1
mass in feed = 1 x 3.4 = 3.4

$$\text{mass ratio} = \frac{23.1}{3.4} = 6.8$$

The value 3.4 is 4 ÷ 1.18 where 1.18 is the estimated concentration which occurred in Run 1 before securing the feed sample. Only on Run 1 is this factor appropriate. No effect of the solute mass in the leak during Run 2 is accounted for in Table 5 mass ratio data.

Table 4

Run 1 Cast Membranes on Dye Fluid
(values in mg/m^3)

Compound	Feed CTHF 10	Permeate Poly Ether/Amide CTHF 11	Permeate Cellulose Acetate CTHF 12	Concentrate CTHF 13	Mass Ratio End/Start	Comments
Bis(2-ethylhexyl) phthalate	4	31	3	51	6.8	mixed rejections, concentrated
Dimethyl phthalate	55	45		290	1.2	positive rejection, concentrated
Di-n-butyl phthalate	1	0.8		6	1.3	positive rejection, concentrated
Butylbenzyl phthalate		1		7	∞	membrane may be source
Diethyl phthalate						not detected
Acenaphthene	3	0.8		7	0.5	rejected and concentrated
Anthracene	0.6			3	0.7	rejected and concentrated
Fluoranthene						not detected
Pyrene						not detected
Naphthalene	0.8				0.0	sorbed or vaporized
Phenanthrene						not detected
Phenol	0.2	0.7	0.4	1	3.6	not rejected, concentrated
Chloroform	19	31	4		1.0	mixed rejected, not concentrated
Toluene	10	11	24		1.7	not rejected
Trichloroethylene		0.6			∞	membrane source
Benzene	2	0.4	1		0.3	rejected, perhaps vaporized
Chlorobenzene						not detected
Ethylbenzene						not detected
Methylene chloride	5	45	4	4	5.7	membrane possibly source
Triphenyl phosphine	5	2	7	10	1.1	container source
Triphenyl phosphine oxide	5	5	10	30	2.3	container source
α-Terepineol	30	20	30	50	1.1	slight rejection

Table 5

Run 2 Dynamic Membrane on Dye Fluid
(values in mg/m^3)

Compound	Feed CTHF 14	Permeate CTHF 15	Concentrate CTHF 16	Mass Ratio	Comments
Bis(2-ethylhexyl) phthalate	2	1	4	0.905	rejected, concentrated
Dimethyl phthalate	170	4		0.02	rejected, not concentrated
Di-n-butyl phthalate	1	1	1	1.0	not rejected, not concentrated
Butylbenzyl phthalate					not detected
Diethyl phthalate		0.05		∞	
Acenaphthene	3			0.0	sorbed
Anthracene	0.7	0.1		0.1	rejected, not concentrated
Fluoranthene	0.1			0.0	sorbed
Pyrene					not detected
Napthalene	0.8			0.0	sorbed
Phenathrene					not detected
Phenol	0.2			0.0	detected
Chloroform	96			0.0	vaporized
Toluene	0.6	0.4	1	0.94	slight rejection
Trichloroethylene	0.6	1		1.22	negative rejection
Benzene					not detected
Chlorobenzene					not detected
Ethylbenzene					not detected
Methylene chloride	5	3	3	0.6	slight rejection, not concentrated
Triphenyl phosphine	10	10	10	1.0	container source
Triphenyl phosphine oxide	10	10	5	0.86	container source
α Terepineol	50	5		0.07	sorbed
2-Mercepto benzthiazole	40	30	200	1.9	slight rejection
1-Cyano-2-benzyloxyethane	60	10	100	0.57	rejected
Benzothizole	200		250	0.34	rejected

Table 6

Run 3 Cast Membranes on Scour Fluid
(values in mg/m^3)

Comments	Feed CTHF 3	Permeate Poly Ether/Amide CTHF 4	Permeate Cellulose Acetate CTHF 5	Concentrate CTHF 6	Mass Ratio	Comments
Bis(2-ethylhexyl) phthalate	9	3	3		.30	mildly rejected, but not concentrated
Dimethyl phthalate		9			∞	permeate possibly contaminated by previous run (CTHF 11)
Di-n-butyl phthalate	4	1			0.1	rejected, but not concentrated
Butylbenzyl phthalate						not detected
Diethyl phthalate						not detected
Acenaphthene	7	0.8			0.05	rejected, but not concentrated
Anthracene	2				0.0	rejected, but not concentrated
Fluoranthene	0.4				0.0	rejected, but not concentrated
Pyrene	1				0.0	rejected, but not concentrated
Naphthalene		0.5			∞	
Phenanthrene						not detected
Phenol		2	3	13	∞	concentrated
Chloroform	18	18	22		0.98	poor rejection
Toluene	0.8	15	29	41	30	concentrated
Trichloroethylene	0.3		0.4	5	2.4	concentrated
Benzene		1	1	6	∞	concentrated
Chlorobenzene			0.7		∞	concentrated
Ethylbenzene				21	∞	concentrated
Methylene chloride	5	6	5	15	1.3	not rejected, but concentrated
Triphenyl phosphine		0.5	2		∞	container source

Table 6 (continued)

Comments	Feed CTHF 3	Permeate Poly Ether/Amide CTHF 4	Permeate Cellulose Acetate CTHF 5	Concentrate CTHF 6	Mass Ratio	Comments
Triphenyl phosphine oxide	5	10	10		1.76	container source
α-Terepineol	10	30			1.42	not rejected
2-Atercapts benzothiazole	10	20	0.5		0.96	not rejected
1-Cyano-2-benzyloxyethane		5			∞	membrane source
Benzothiazole	30	2		600	2.4	rejected, concentrated
Lauric Acid	400			3000	0.9	rejected, concentrated
Myristic Acid				1000	∞	concentrated
Palmitic Acid				1000	∞	concentrated

Table 7

Run 4 Dynamic Membrane on Scour Fluid
(values in mg/m^3)

Compound	Feed CTHF 7	Permeate CTHF 8	Concentrate CTHF 9	Mass Ratio	Comments
Bis(2-ethylhexyl) phthalate	9			0.0	sorbed
Dimethyl phthalate					not detected
Di-n-butyl phthalate	3			0.0	sorbed
Butylbenzyl phthalate					not detected
Diethyl phthalate					not detected
Acenaphthene	7			0.0	sorbed
Anthracene					not detected
Fluoranthene					not detected
Pyrene					not detected
Naphthalene					not detected
Phenanthrene	2			0.0	sorbed
Phenol			1	∞	source possibly in residual of previous fluid
Chloroform	34			0.0	vaporized
Toluene	0.8	0.7	0.5	0.84	rejected mildly, possibly vaporized
Trichloroethylene		2		∞	
Benzene		2	0.7	∞	
Chlorobenzene					not detected
Ethylbenzene					not detected
Methylene chloride	4	5	2	1.11	negative rejection
Triphenyl phosphine		5		∞	container source
Triphenyl phosphine oxide	2	30	5	11.6	container source
α-Terepineol	25			0	sorbed
2-Mercapto-benzothiazole	10			0	sorbed
Benzothiazole	40	5	100	0.76	rejected, concentrated
Lauric acid			100	∞	concentrated
Palmitic acid			400	∞	concentrated
Stearic acid			200	∞	concentrated

A value of one in mass ratio indicates a consistent total solute mass. Values greater or less than one imply that the mass is estimated to have increased or decreased. Increases in mass imply a source of solute either from carryover from a previous run or desorption from the membrane or equipment. Since care was taken to use only stainless steel and teflon in the system, and the membranes were flushed reasonably well the latter source was as small as was practical. The plastic (polyethylene) covers on the tanks could have served as sources for phthalates when the condensing vapors dripped into the tank. The possibility of carryover from the previous run are acknowledged in the comments on the tables.

Many of the solutes subject to analysis expected in the concentrate were not detected there. This is especially true of the base neutral compounds in the dynamic membrane tests (Table 5 and 7). These compounds are not highly volatile, but may have been sorbed into the apparatus or rendered not extractable for analysis. The more volatile compounds chloroform and benzene probably vaporized. Toluene may have been sourced from the cellulose acetate and poly(ether/amide) membranes and as such the rejection may be masked.

The number and level of concentration of toxic organic compounds was low in all runs. Because of this and the analytical inaccuracy (±100 percent) the calculation of rejection is not meaningful.

However, if either decreased permeate concentrations or increased concentrations of concentrate can be used to signal positive rejection, forty-three of fifty-one show positive indication and eight indicate corroborating data for low rejection. Chloroform, toluene, trichloroehtylene, and methylene chloride all show a somewhat consistent trend to low rejection. The evidence for rejection is mixed for phenol and di-n-Butyl phthalate. The remainder of compounds have at least some evidence in each set of data to indicate positive rejection. These observations are actually stronger than is actually substantiated by the data, but represent the trends which are apparent.

A few additional organic compounds detected without the use of standards are identified in Appendix C. Those most prominent are the acid complement to certain detergents (lauric acid, myristic acid, palmitic acid) which were noted almost exclusively in the concentrated samples. Benzothiazole was detected in three runs and was rejected effectively.

Metals

Metal analyses for toxic pollutants and other metals were performed by Monsanto Research Corporation. Analysis for arsenic was performed by conventional atomic absorption, the others were analyzed in neat and digested samples. The neat analysis results were suspected of showing an effect due to organic loading. The digested samples do not show such effects. Raw analysis for the digested samples has been corrected for metals in dilution water and reagent acid which were added during digestion. The results, as corrected are shown in Table 8. Very low levels of most toxic metals are notable.

Table 8 Metal Analysis

Concentration in Streams (mg/m^3)

Metal	Plant CTHF-1	Apparatus CTHF-2	Feed CTHF-3	Permeate, PEA CTHF-4	Permeate, CA CTHF-5	Concentrate CTHF-6	Feed CTHF-7	Permeate, DM CTHF-8	Concentrate CTHF-9
Aluminum	106	95	794	77	164	3,690	1,270	1,260	4,890
Antimony	<0	<0	38	23	70	364	103	79	308
Arsenic	<1	<1	19	<1	1	160	35	5	~14
Barium	98	78	82	-	8	578	118	6	348
Beryllium	-	-	-	-	-	-	-	-	-
Boron	553	308	47,200	11,900	8,900	81,000	56,000	31,000	81,000
Cadmium	-	-	6	6	5	38	9	11	110
Calcium	13,300	16,100	15,900	608	892	113,700	15,200	1,078	63,500
Chromium	206	355	306	310	286	775	350	390	555
Cobalt	-	2	15	-	0	65	11	13	45
Copper	36	178	72	6	14	738	74	48	622
Iron	965	269	445	212	119	2,800	332	212	1,900
Lead	51	82	263	112	223	602	262	276	762
Magnesium	5,060	6,680	9,260	194	320	71,950	6,154	362	28,750
Manganese	256	146	356	20	22	2,600	716	22	2,860
Molybdenum	-	-	16	-	28	118	21	55	190
Nickel	-	79	61	-	29	405	127	137	393
Phosphorus	-	1,320	3,926	95	526	33,200	4,830	1,100	23,600
Silicon	13,150	17,800	17,300	2,200	2,250	29,600	20,600	7,200	22,000
Silver	-	-	11	-	11	83	31	47	169
Sodium	57,800	103,000	378,000	11,720	23,400	1,672,000	610,000	242,000	1,544,000
Strontium	130	153	142	-	-	1,040	138	-	560
Tin	-	-	64	-	-	520	68	-	260
Titanium	-	-	35	1	15	75	21	5	59
Vanadium	19	30	69	5	9	480	55	29	230
Zinc	216	202	106	-	8,180	3,120	46	-	6,146

Table 8 (continued)

Metal	Feed CTHF-10	Permeate, PEA CTHF-11	Permeate, CA CTHF-12	Concentrate CTHF-13	Feed CTHF-14	CTHF-15	Concentrate CTHF-16
Aluminum	431	165	116	10,900	1,090	640	2,900
Antimony	120	65	54	208	124	98	248
Arsenic	35	15	<1	221	2	<1	9
Barium	62	-	-	398	194	-	478
Beryllium	-	-	34	-	-	-	-
Boron	2,500	430	1,160	3,950	772	683	1,232
Cadmium	12	11	39	30	10	25	60
Calcium	16,300	618	252	113,100	25,500	532	70,100
Chromium	55	350	186	575	415	346	835
Cobalt	19	9	37	61	13	13	59
Copper	358	26	32	3,040	10,600	82	35,400
Iron	328	192	55	950	300	105	1,240
Lead	362	266	263	542	362	333	982
Magnesium	11,350	94	66	81,800	12,950	200	41,150
Manganese	276	12	36	1,860	696	10	396
Molybdenum	56	39	60	112	66	124	358
Nickel	145	127	-	405	111	149	525
Phosphorus	7,200	375	366	49,400	49,400	3,870	140,800
Silicon	23,400	120	3,010	39,200	11,600	12,900	19,800
Silver	51	37	-	85	39	53	95
Sodium	185,000	11,600	7,190	894,000	528,000	90,000	1,247,000
Strontium	144	-	-	1,000	258	-	718
Tin	60	-	60	200	50	12	120
Titanium	17	5	1	35	9	11	27
Vanadium	86	15	-	530	96	21	290
Zinc	6,780	-	-	4,946	6,190	-	12,390

The rejection of metals in the proces water is difficult to estimate in most cases due to the low concentration levels. As has already been mentioned, digestion of metal samples was performed, with the result that metal addition from nitric acid and distilled water occurred. In many cases, the metal addition was of the same magnitude as the total concentration in the feed sample. Thus the correction applied was as large as was the metal inclusion. Since membranes have shown an excellent rejection[3] for metals regardless of form (ionic, complexed, etc.) the anticipated level in a permeate is at least an order of magnitude lower than the feed concentration. In such a case the permeate analysis is subject to very large errors due to ordinary uncertainty. For this reason the results for rejection have been separated into three groups.

Group I (results shown in Table 9) contains the data for which the feed and permeate level is sufficiently high to provide a normal estimate of rejection. The criterion used is that the feed content is at least five times that amount added during digestion of the sample.

TABLE 9. Percent Rejection of Metals by Hyperfiltration: Group I Results
(Normal Confidence)

Metal Toxic	Poly(ether/amide)		Cellulose Acetate		Dynamic Membrane	
Pollutants	Scour	Dye	Scour	Dye	Scour	Dye
Arsenic	98	75	98	>98	94	>69
Copper		97		96		>99
Zinc		100		100		100
Other Metals						
Aluminum	96		90		1	60
Barium	100	100	96	100	97	100
Boron	88	92	92	72	64	32
Calcium	98	98	98	>99	97	99
Iron	70		87			
Magnesium	99	99	98	99	97	99
Manganese	97	98	97	95	98	98
Phosphorus	98	97	95	97	89	96
Silicon	94	100	94	95	81	0
Sodium	98	97	97	98	77	92
Strontium	100	100	100	100	100	100
Tin	100	100	100	<0	100	88
Titanium	>99		74			
Vanadium	97	92	94	100	65	90

Omission from this table implies a low value of feed concentration. See text for details.

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[3] Brandon, C. A., J. J. Porter, and D. K. Todd, "Hyperfiltration for Renovation of Composite Wastewater at Eight Textile Finishing Plants," Final Report, EPA Grant 802973.

Group II (results shown in Table 10) contains the data for which the feed has less than five times but more than twice the amount added during digestion of samples. Rejections thus obtained are subject to greater uncertainty than normal and the values should be treated as an indication of rejection.

TABLE 10. Percent Rejection of Metals by Hyperfiltration: Group II Results (Reduced Confidence Level)

Toxic Metals	Poly(ether/amide) Scour	Poly(ether/amide) Dye	Cellulose Acetate Scour	Cellulose Acetate Dye	Dynamic Membrane Scour	Dynamic Membrane Dye
Copper	96	97*	91	96*	55	99*
Lead	75	44	32	46		25
Other Metals						
Aluminum	96*	78	90*	77	1*	60*
Cobalt		70		<0		
Iron	70*	60	87*	92	55	80*
Titanium	>99*	85	74*	97	88	

Omission from this table implies a near absence in feed. See text for details.

*Values marked are higher confidence data from Table 9.

Group III contains the data having feed solute mass less than twice that added in digestion. For these data, the uncertainty in feed and product is such that the respective values of concentration may overlap resulting in about as many negative as positive calculated rejections. These data are not presented in rejection form because they are not considered to be meaningful.

In all the data of Tables 9 and 10 the curve of rejection as dependent on permeate and feed concentration ratio has been employed from Figure 3.

According to the foregoing criteria, some metals were present in such low concentration that the analysis cannot be expected to provide even an indication of the rejection. These metals are Antimony, Beryllium, Cadmium, Chromium, Nickel, and Silver from the toxic pollutant list. In some runs zinc and lead also were below the concentration criterion. Arsenic was present in low levels (20 mg/m^3) but was analyzed without digestion such that analysis is expected to be accurate. Some copper and zinc levels were high enough to qualify for normal rejection assessment. These appear to be the only toxic metals present in the process water and occur only in the dye effluent.

Despite the limited data for rejection of metals obtained in this effort, membranes have historically shown excellent rejection for metals. This trend is corroborated by the data in Table 9. Three unusually low or negative rejection data are shown in the "other metal" list: aluminum on the scour with the dynamic membrane, silicon on the dye with the dynamic membrane, and tin on the dye with the cellulose acetate membrane. In each of these cases reference to the concentrate data of Table 8 shows that the element was concentrated. Therefore, it is considered that some anomaly of analysis is involved and that probably the rejections are not as low as indicated.

Bioassays

The values of LC_{50} (or EC_{50}) obtained from each sample may be heuristically related to the concentration of an unknown substance. The concentration of that unknown substance which produces 50% mortality is expected to be a reasonably repeatable value, say C*. When a volume of fluid contains LC_{50} of a sample and $(1-LC_{50})$ of diluent water the concentration of unknown substances is C*. Also one can use this fact to determine the concentration (C) from $C \cdot LC_{50} = C^*$. Therefore, the concentration (C) of toxic substance is inversely proportional to the value of LC_{50}.

Obviously the foregoing statement applies to the simplest, single toxicant solution. However, if the membrane is not highly selective in rejection for a multicomponent mixture a very similar result would obtain for comparison of toxic effects of feed and concentrate, etc. Therefore, the data for LC_{50} have been used to calculate relative values for the implied concentration of toxic substance to enable the calculation of membrane rejection. The bioassay tests results for LC50 are presented together with the implied concentration of toxicant in Table 11. The values for concentration of toxicant are simply 100 divided by its respective LC_{50} value. The information in Table 11 is organized in the order of actual test sequence which is different from the sample numbering sequence.

Values of implied concentration from Table 11 are used to calculate the rejection again using Figure 3 as a basis. All rejections of toxicant concentration are substantial as shown in Table 12. The toxic level of each concentrate was 5 to 11 times higher than that of the feed, providing consistent evidence of membrane separation. A mass ratio of the implied concentration of supposed toxicant is presented in Table 13. Mass ratio is the combined mass of solute in permeate and concentrate divided by the mass of solute in the feed. A sample calculation is provided in Appendix B. The results are reasonably consistent (mass ratio ≈ 1), ranging from 0.65 to 1.55.

The rejections shown in Table 12 are of considerable interest. As already mentioned the bioassay results are consistent in showing reduced toxic effect in permeates and corroborating increased toxic effects in the concentrate. The rejection of material toxic to the daphnids is uniformly lower than that of toxic to Fathead minnows. The toxicant rejections are opposite to the rejection of inorganic salts. That is, the dynamic membrane produces superior separation to the cellulose acetate which is superior to the poly(ether/amide) on toxic substances. By contrast the inorganic (salt) rejection exactly counters the ordering. Simply stated this only means the

Table 11

Lethal Concentration and Implied Toxicant Concentrations

Fluid and Type	Sample No.	96 Hour Minnows		48 Hour Daphnia	
		LC_{50} % Solution	Implied Concentration No Units**	LC_{50} % Solution	Implied Concentration No Units**
Run 1					
Dye-feed	10	9.7	8.5***	33.5	2.5***
Dye-PEA permeate	11	82	1.2	60 to 100	1 to 1.7
Dye-CA permeate	12	>100	<1.	60 to 100	1 to 1.7
Dye-concentrate	13	1.6	62.	4.1	24.
Run 2					
Dye-feed	14	25	4.	49	2.0
Dye-DM permeate	15	NAT*	0.	80	1.2
Dye-concentrate	16	5.3	19.	17	5.9
Run 3					
Scour-feed	3	16	6.	26	3.8
Scour-PEA permeate	4	28	3.6	53	1.9
Scour-CA permeate	5	>100	<1.	42	2.4
Scour-concentrate	6	1.5	67.	5.1	20.
Run 4					
Scour-feed	7	13	7.7	25	4.
Scour-DM permeate	8	NAT*	0.0	>100	1.
Scour-concentrate	9	2.0	50.	9.9	<10.

*NAT - no acute toxicity

**by Implied Concentrations in headings

$$\text{Implied Concentration} = \frac{100}{LC_{50}}$$

***Values lowered due to concentration of sample removed for feed

TABLE 12. Rejection of Toxicity by Hyperfiltration

Scour Fluid	% Rejection	
Membrane	Daphnia Toxicant	Fathead Minnow Toxicant
Dynamic ZrO/PAA	>88	100
Cellulose Acetate	55	>92
Poly(ether/amide)	68	60
Dye Fluid	% Rejection	
Membrane	Daphnia Toxicant	Fathead Minnow Toxicant
Dynamic ZrO/PAA	60	100
Cellulose Acetate	62 to 82	96
Poly(ether/amide)	62 to 82	95

Data in this table are obtained from the procedure

$$C.R. = \frac{\text{Implied concentration of permeate (from Table 11)}}{\text{Implied concentration of feed (from Table 11)}}$$

C. R. is the concentration ratio used as abscissa for Figure 3. The rejection is read as the ordinate of Figure 3.

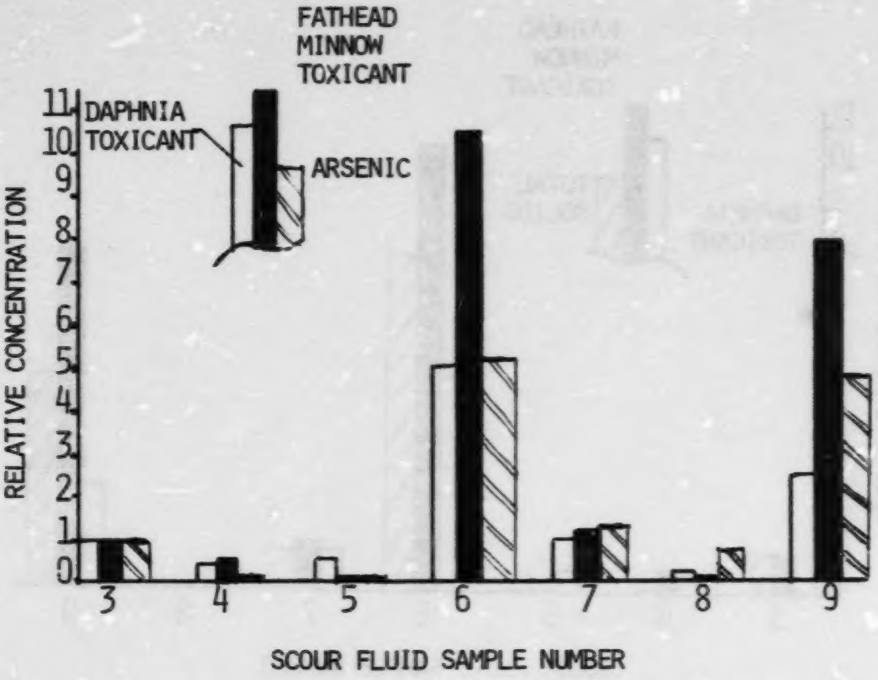
TABLE 13. Mass Ratio[a] of Toxicants

Run 1 Cast Membranes on Dye Fluid	
Toxicant to	Mass Ratio (final/initial)
Fathead minnows	0.94
Daphnids	1.315
Run 2 Dynamic Membrane on Dye Fluid	
Toxicant to	Mass Ratio (final/initial)
Fathead minnows	1.28
Daphnids	1.23
Run 3 Cast Membranes on Scour Fluid	
Toxicant to	Mass Ratio (final/initial)
Fathead minnows	1.55
Daphnids	1.08
Run 4 Dynamic Membrane on Scour Fluid	
Toxicant to	Mass Ratio (final/initial)
Fathead minnows	1.17
Daphnids	0.65

[a] A mass ratio calculation example is shown in Appendix B.

membranes developed to achieve high salt rejection for desalination applications do not necessarily have proportional rejections of toxic (presumably non-electrolytic) compounds.

In an attempt to determine cause and effect, the toxicant concentration profile from Table 11 may be compared with measured concentrations of substances. Three of the best fit profiles are shown in Figures 4 through 6. The relative toxicant concentrations are shown for the Daphnia and Fathead minnows as compared with total solids, arsenic, and copper in the succeeding figures. None of the organic toxic pollutants has a concentration pattern remotely similar to the bioassay results. Arsenic and total solids shown patterns resembling the bioassay results, while copper fails badly



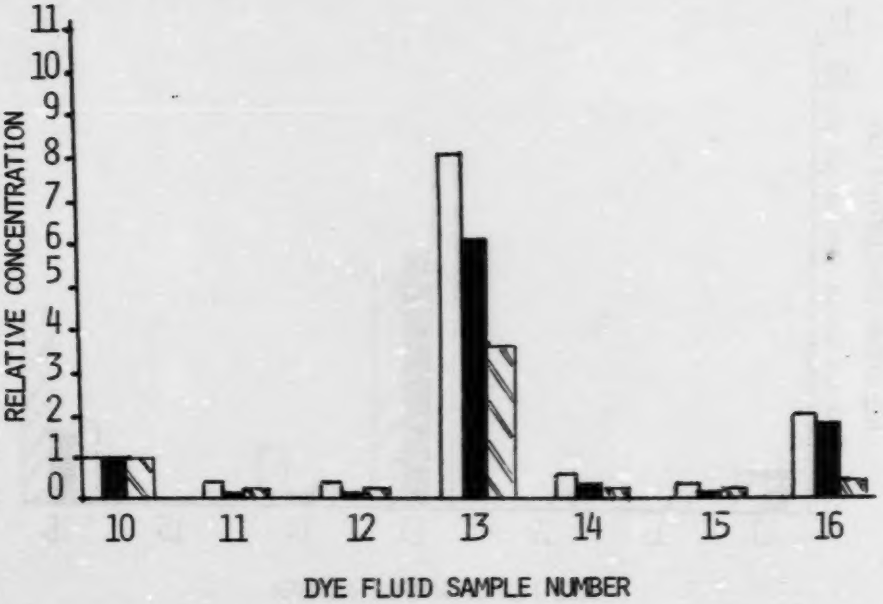


FIGURE 4 RELATIVE CONCENTRATIONS OF TOXICANTS AND ARSENIC

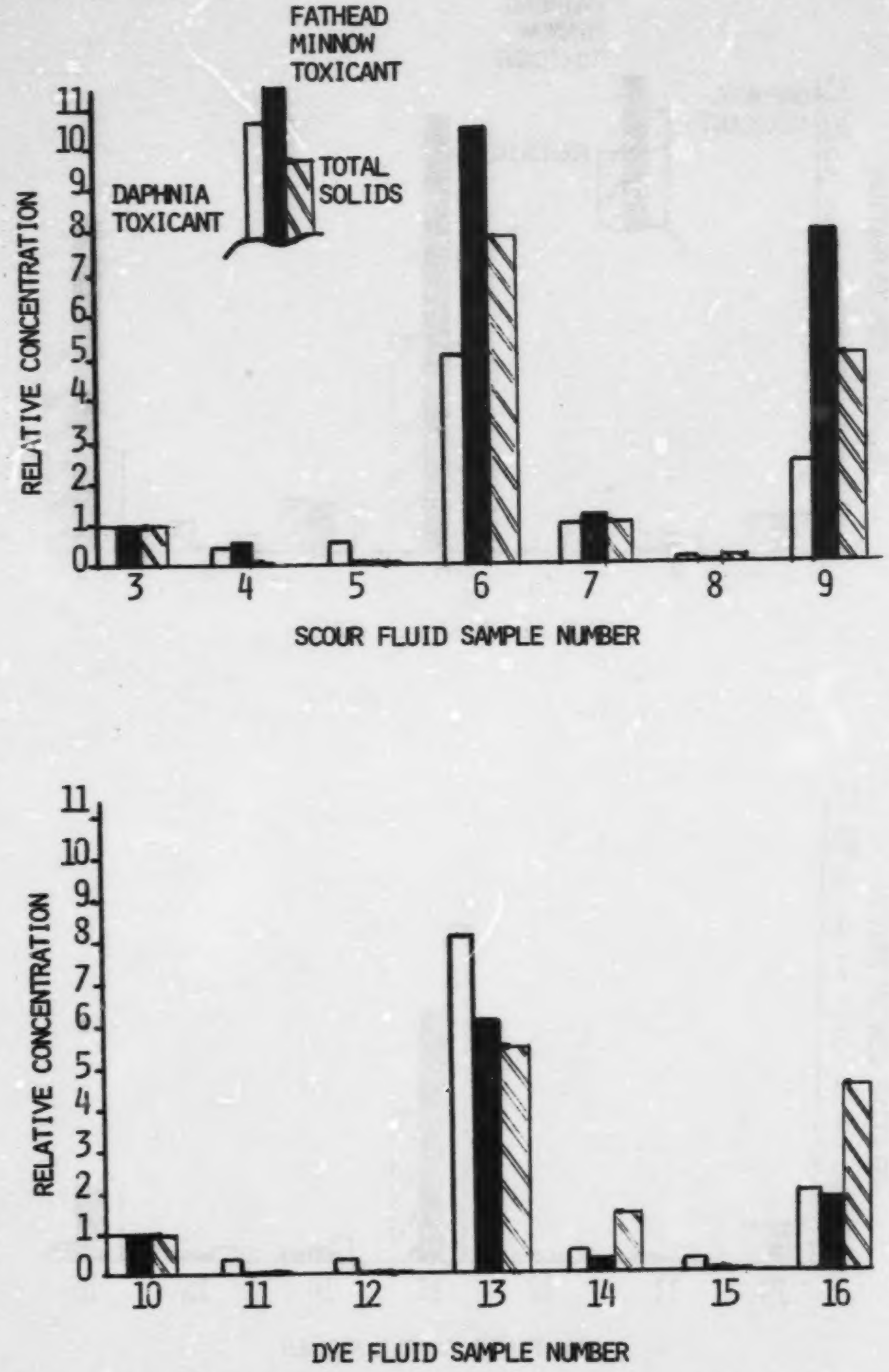


FIGURE 5 RELATIVE CONCENTRATIONS OF TOXICANTS AND TOTAL SOLIDS

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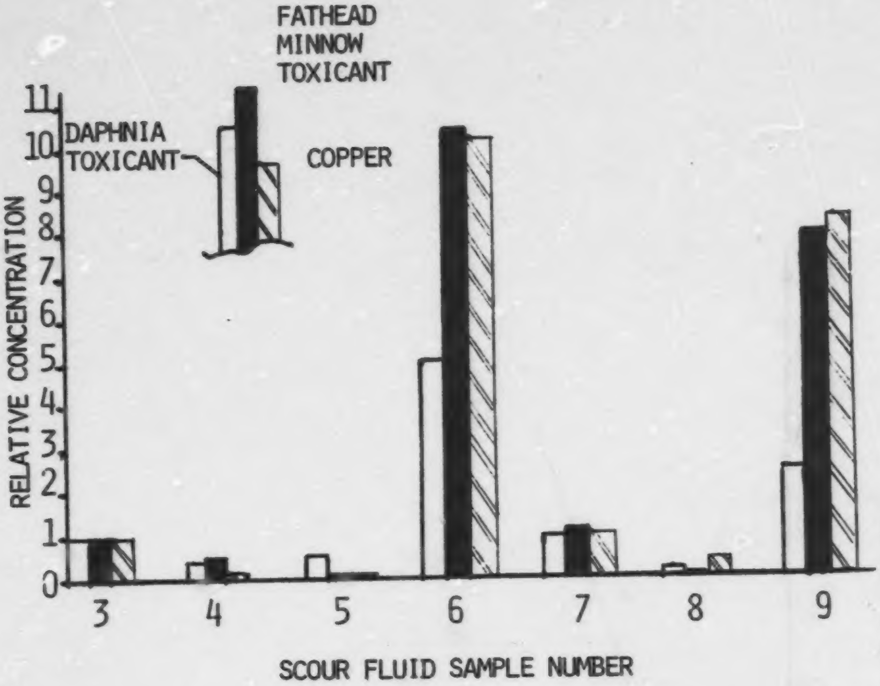


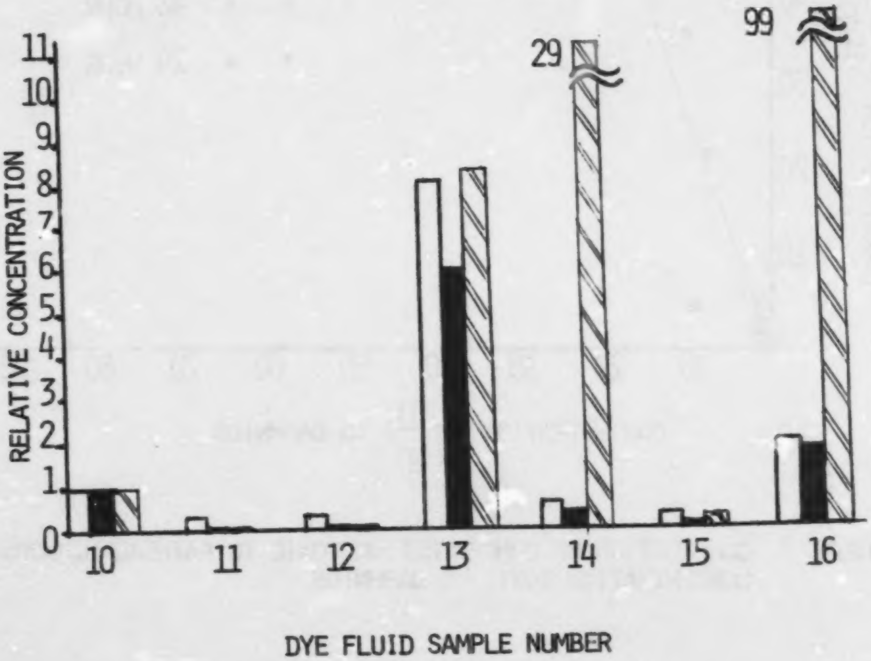


FIGURE 6 RELATIVE CONCENTRATIONS OF TOXICANTS AND COPPER

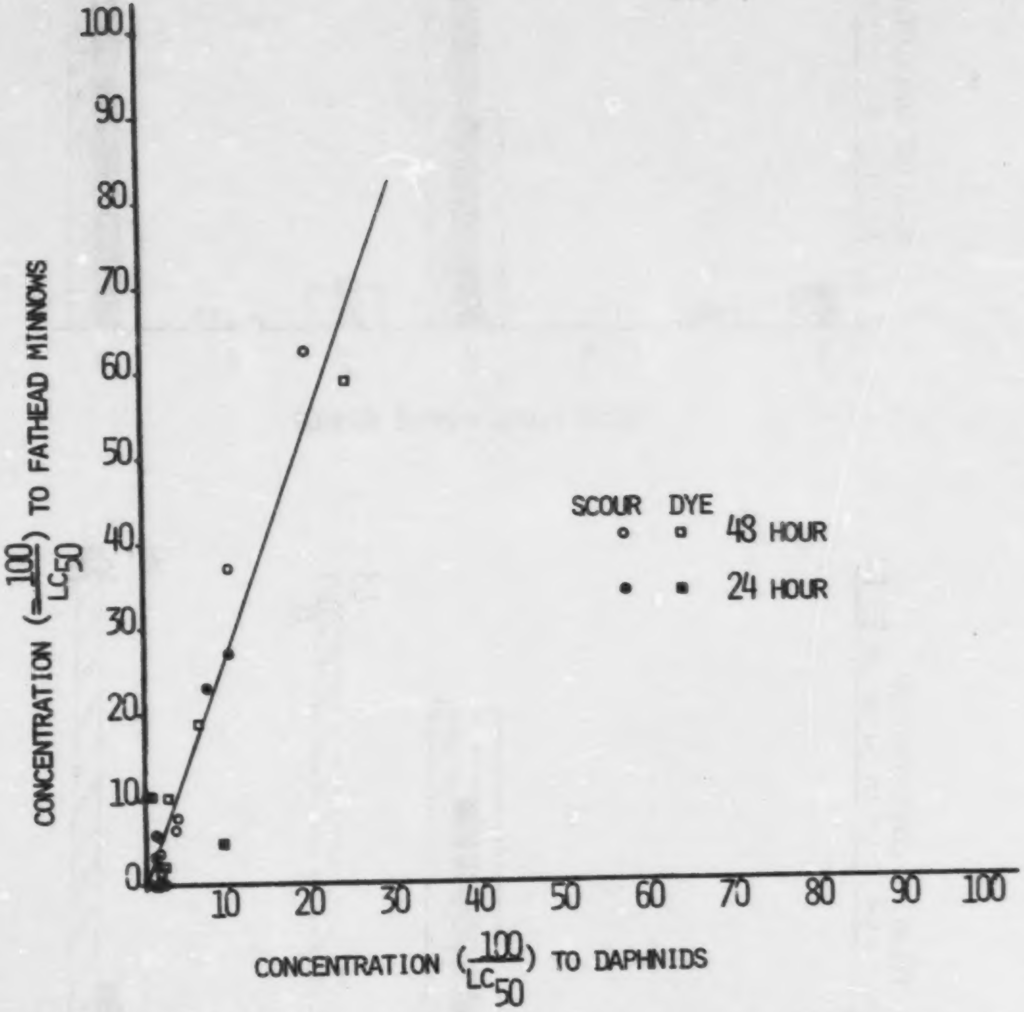


FIGURE 7 CORRELATION OF CONCENTRATION TOXIC TO FATHEAD MINNOWS WITH CONCENTRATION TOXIC TO DAPHNIDS

for the samples 14 and 16. The run in which samples 14 and 16 were taken contained a much larger copper content than any of the other runs and yet did not show proportionally high toxic effects. The presence of a dye containing complexed copper could account for this result. It is doubtful that the low values of arsenic could be toxic. Therefore, no simple cause/effect can be determined; and, further it is likely that one or more of the gross, non-analyzed compounds served as toxicant assuming its separation reasonably paralleled that of the metals or total solids.

A correlation of toxic concentration to Fathead minnows and to Daphnids may be investigated. A plot of implied toxicant concentration for minnows versus the concentration for daphnids is shown in Figure 7. Alternatively, Figure 7 may be viewed simply as a plot of reciprocal LC_{50} data. There is a high correlation coefficient of 0.94 suggesting that for this fluid a measurement of either individual bioassay would have supplied essentially the same information. A plot of concentration at which no effects were observed (reciprocal EC_0) is similar but shows a far greater range and scatter. The Daphnia were more sensitive than the minnows to the test fluid, judged by nine of fourteen values in Table 11.

Rat toxicity and bacterial mutagencity tests produced no effective response. The concentrates from each run produced responses at about 90 percent dilution suggesting that the feed may also have been marginally cytotoxic. Neither the feed nor permeates produced position cytotoxicity results. Appendix C contains the detailed results.

Correlation of Rejection in Single-Solute Solutions with Solute Solubility Parameter

Hyperfiltration rejection of organic nonelectrolytes in single-solute has often been correlated with the molecular weight of the solute although for low molecular weight compounds the correlation is sometimes poor, especially for cellulose acetate membranes. The dependence of rejection on solute solubility parameter has been demonstrated using published hyperfiltration results. Appendix D describes the results of this correlation.

If this correlation is satisfactory, or can be developed into a reliable model, it would greatly reduce the experimental work required to characterize the effectiveness of a membrane to reject toxic pollutants. Rejections of a few solutes could be determined for a solution-membrane system and the rejection of other solutes estimated.

TEST DESCRIPTION

Fluid samples were obtained at the overflow of the first washer on the Küsters dye range at the La France Industries plant (see Figure 8). The effluent was collected in a plastic pail fitted with a 40-meter rubber hose connected to a 80ℓ/min centrifugal type transfer pump. The pail and entire hose had been previously used extensively with the fluids from the range. Non-stainless steel parts of the pump hardware were replaced with stainless steel. The pump was all stainless steel with ceramic seals. The fluid was passed through a one-micron polypropylene cartridge filter. New filters were used for the bleach (scour) acquisition. The fluid line was purged before each new fluid acquisition.

All fluid lines and wetted parts in the test system were Teflon, stainless steel or ceramic except one line having a rubber tube joining two steel tubes in a non-flowing channel used as a connection to a suction pressure protection device. The feed and permeate tanks were cleaned with a commercial cleaner used to clean becks at La France. Following this the tanks and the skid-mounted pump station were flushed thoroughly for one-half hour in 1 M NaOH and rinsed with plant water, until no pH elevation was present. The tanks were covered with new polyethylene film to assist in vapor and volatile retention and to prevent entrance of the airborne lint.

Pressure, temperature, and flow to the membrane were controlled at the skid mounted pump station. Conditions were maintained during the runs at the values shown in Table 14. The range of pressure and temperature shown in Table 14 was selected in the dynamic membrane tests to allow stable operation at a rate to achieve a reasonable time to acquire samples. All values are approximate and varied slightly from the conditions listed. The other membranes were operated at conditions determined in concert with the manufacturer.

TABLE 14. Operating Conditions Observed

Fluid	Membrane	Temperature (°C)	Outlet Flow (ℓ/min)	Inlet Pressure (MN/m^3)
Dye	PEA-CA	40	16	2.8 (400 psi)
Scour	PEA-CA	40	16	2.8 (400 psi)
Dye	Dynamic	70	16	4.5 (650 psi)
Scour	Dynamic	77	16	5.9 (850 psi)

The dye test fluids are the wash water obtained while using a dye pad formulation for direct dyeing cotton. These dye pad formulations contain

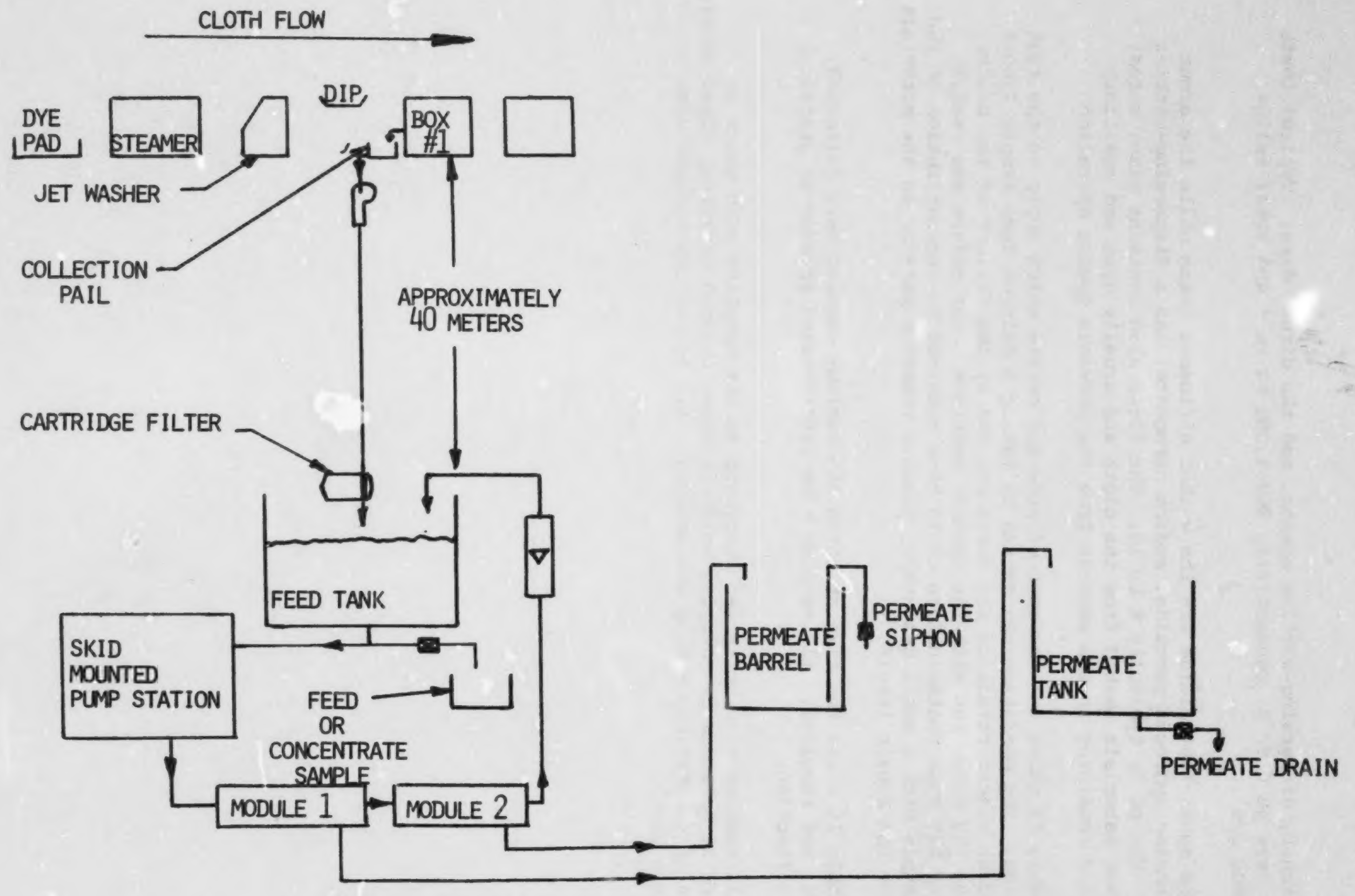

CLOTH FLOW
DYE PAD
STEAMER
DIP
BOX #1
JET WASHER
COLLECTION PAIL
APPROXIMATELY 40 METERS
CARTRIDGE FILTER
FEED TANK
SKID MOUNTED PUMP STATION
FEED OR CONCENTRATE SAMPLE
MODULE 1
MODULE 2
PERMEATE BARREL
PERMEATE SIPHON
PERMEATE TANK
PERMEATE DRAIN

FIGURE 8 SCHEMATIC OF FLUID ACQUISITION AND OPERATIONS

a thickener, dispersing-wetting agents, and the direct dyes. Typical tests fluids have pH 6 to 8, conductivity 200-1,000 μS cm^{-1} and total solids 400-2,800 g/m^3.

The scour test fluids are the washer effluents taken while the scour pad contains hydrogen peroxide, sodium carbonate, and a dispersing-wetting agent. The pH is typically 8 to 10. The fluid also contains size, motes, and other materials washed from the cloth and usually dyes and auxiliary chemicals remaining in the washers from the previous dyeing operation.

Table 15 shows the sequence of runs and events which apply to the test operation. The operation was marred by taking a delayed feed sample (about 15 percent concentrated) on the first run and by the failure of the solder joint on the scour run with the dynamic membrane. The module was readily repaired but some contamination could have occurred in reconstitution of the feed sample with a small gear-type, plastic transfer pump or in the materials used for the repair itself.

Table 16 shows the time at which the various samples were collected, shipped, and received. All samples were refrigerated as soon as practical after collection.

All samples were collected according to the sampling plan which is included as Appendix E. Samples were withdrawn through stainless steel tubes; the use of plasticized tubing was avoided. All collection barrels were stainless steel.

Table 15

Summary Log of Activities

Date	Time	Activity
6/01/78	1430	Obtain "clear" water sample from range: 280ℓ at 60°C drawn through 1 micron polypropylene filter. Previous dye formula was 9127.
6/01/78	1450	Operate membranes (PEA and CA) at 300 psi. 53°C feed cooled at 46°C by heat exchanger.
6/01/78	1500	Stop, apparatus blank run. Take sample half from permeate, half from concentrate.
6/01/78	1545	Drain all tanks.
6/01/78	1600	Obtain dye batch: dye formula 9204. 654ℓ. Allow to cool overnight.
6/02/78	1114	Start unit. Discard first liter of product.
6/02/78	1140	Stop unit at 15.6% recovery. Obtain slightly concentrated feed sample.
6/02/78	1215	Resume operation.
6/02/78	1312	Stop unit, obtain samples at 90% recovery. Drain tanks.
6/09/78	1100	Obtain batch and feed sample from dye formula 1211, partly unfiltered batch. Install dynamic membrane 0.3 m^2. Start operation at $4MN/m^2$ (580 psi) with poor rejection of color.
6/09/78	2230	Return permeate in clean glass bottle to feed. Permeate has cleared.
6/11/78	2215	Stop small leak from plumbing.
6/12/78	0730	Stop operation, obtain samples. Approximate 80% recovery.
6/12/78	0830	New polypropylene feed filter installed. PEA and CA membranes connected after flushing. Scour feed batch obtained of 429ℓ. Sample taken.
6/12/78	1107	Start run on scour.
6/12/78	1213	Stop run at 83% recovery. Obtain samples.
6/12/78	2000	Obtain scour batch for dynamic membrane; 465ℓ.
6/13/78	1000	Connect dynamic membrane, gather feed sample, start operation.
6/14/78	0400	Module failure - soldered joint failed.
6/14/78	0800	Repair module.
6/14/78	1130	Return fluid to feed using 2m vinyl hose and plastic gear pump. Restart test.
6/17/78	2300	Stop test at 80% recovery, obtain samples.
6/18/78	0100	Obtain plant water blank sample.

TABLE 16. Sample Disposition Log

Sample Number	Date - Hour Taken		Date Shipped Chemical	Fish	Rat	Date Received Chemical	Fish	Rat
1	6/18 -	0100	6/19	-	-	6/20	-	-
2	6/01 -	1500	6/05	-	-	6/06	-	-
3	6/12 -	0830	6/13	6/14	6/19	6/14	6/15	6/20
4	6/12 -	1300	6/13	6/20	6/19	6/14	6/21	6/20
5	6/12 -	1300	6/13	6/20	6/19	6/14	6/21	6/20
6	6/12 -	1300	6/13	6/14	6/19	6/19[a]	6/15	6/20
7	6/12 -	1000	6/13	6/14	6/19	6/14	6/15	6/20
8	6/17 -	2300	6/19	6/20	6/19	6/20	6/21	6/20
9	6/ -	2300	6/19	6/20	6/19	6/20	6/21	6/20
10	6/02 -	1200	6/05	6/08	6/19	6/06	6/09	6/20
11	6/02 -	1300	6/05	6/08	6/19	6/06	6/09	6/20
12	6/02 -	1300	6/05	6/08	6/19	6/06	6/09	6/20
13	6/02 -	1300	6/05	6/08	6/19	6/06	6/09	6/20
14	6/09 -	1200	6/13	6/14	6/19	6/14	6/15	6/20
15	6/12 -	0730	6/13	6/14	6/19	6/14	6/15	6/20
16	6/12 -	0730	6/13	6/14	6/19	6/14	6/15	6/20

[a] Note length of time between date shipped and date received.

REFERENCES

1. G. D. Rawlings and Max Samfield, "Source Assessment: Textile Plant Wastewater Toxics Study Phase I, EPA 600/2-78-004h, March, 1978.

2. "Dyes and the Environment," ADMI Report, Volume II, September, 1974.

3. C. A. Brandon, J. J. Porter, and D. K. Todd, "Hyperfiltration for Renovation of Composite Wastewater at Eight Textile Finishing Plants," Final Report, EPA Grant 802973.

APPENDIX A

Infrared Spectra of Sample and Process Chemical Residues

Infrared spectra were obtained of the evaporation residues of the hyperfiltration solutions, i.e., feed, permeate, and concentrate; the scour chemicals; and the auxiliary dye bath chemicals. A measured volume of each solution was evaporated to apparent dryness in an oven at ca. 105°C. The larger residues were scraped from the evaporating dishes and stored in vials. The permeate residues were quite small and firmly attached to the evaporating dishes, so they were softened with a drop or two of water and the slurry scraped into a mortar and the water evaporated again by placing the mortar in the oven. The spectra were obtained with a Perkin-Elmer 317 infrared spectrophotometer using the KBr pellet technique. In the case of permeate samples, the KBr was added to the mortar and ground to a fine power to incorporate the residue in the pellet.

Table A1 identifies the samples and describes the appearance of the residues. A film like material was observed in some residues, presumably composed of the high molecular weight thickener and/or size removed by the scour. This observation is identified by the film notation. Table A2 identifies the process chemicals, other than dyes.

TABLE A1. Hyperfiltration Samples and Residues Characteristics

CTHF No.	Identification	Total Solids (mg/m^3)	Absorbance 410 m	Description of Residue
1	Plant Water	15,000	0	Not determined
2	Apparatus Water	43,000	.005	Brown powder
3	Scour-1, feed	730,000	.050	Light yellow powder
4	Scour-1, PEA permeate	105,000	0	Colorless deposite
5	Scour-1, CA permeate	32,000	0	Colorless deposite
6	Scour-1, Concentrate	6,020,000	.50	Light brown, film
7	Scour-2, feed	870,000	.03	Cream powder
8	Scout-2, DM permeate	205,000	.01	Colorless powder
9	Scour-2, concentrate	3,840,000	.15	Light brown, film
10	Dye-1, feed	462,000	.10	Green-brown particles
11	Dye-1, PEA permeate	15,000	0	Colorless deposite
12	Dye-1, CA permeate	45,000	0	Colorless deposite
13	Dye-1, concentrate	2,670,000	.65	Dark brown, film
14	Dye-2, feed	76,000	2.0	Dark red, powder and film
15	Dye-2, DM permeate	60,000	0	Slightly pink powder
16	Dye-2, concentrate	2,160,000	7.75	Dark red, film

TABLE A2. Process Chemicals

Identification	Description	Occurrence in Process
Sodium carbonate	Colorless solution	Scour bath
Hydrogen peroxide	Colorless solution	Scour bath
Size	Colorless powder	May wash off cloth in scour
Thickener	Yellow powder	Dye bath
Dispersing Wetting Agent-1	Brown-orange solution	Dye bath
Dispersing Wetting Agent-2	Yellow solution	Dye bath and scour

The infrared spectra are presented in Figures A1 - A22.

The hyperfiltration solutions are multicomponent and the infrared spectra of their residues are complicated. Little information about the relative passage of the components through the hyperfilters is obvious. The spectra have been analyzed using two simple methods. First, the relative absorbances, $A\lambda$, of the strongest three peaks are compared for each hyperfiltration experiment. Selectivity of the membranes with respect to the ir-active components is indicated if the relative absorbance of the peaks differ in the feed and permeate and/or feed and concentrate. The results of this analysis are provided in Table A3. The comparison of $A_{9.0}/A_{7.1}$ for the scour experiments and $A_{9.1}/A_{7.0}$ for the dye experiments indicates membrane selectivity of the ir-active components. The observed appearance and disappearance of other peaks also indicated selectivity.

TABLE A3. Relative Absorbance of Strong Infrared Maxima

CTHF No.	Identification	Relative	Absorbance	Comments
		$A_{9.0}/A_{7.1}$	$A_{6.2}/A_{7.1}$	
10	Dye-1, feed	1.8	0.90	
11	Dye-1, PEA permeate	1.6	1.0	
12	Dye-1, CA permeate	(essentially	KBr spectrum)	
13	Dye-1, concentrate	2.8	1.0	
		$A_{9.0}/A_{7.1}$	$A_{6.2}/A_{7.1}$	
14	Dye-2, feed	3.3	1.6	
15	Dye-2, DM permeate	1.0	.78	
16	Dye-2, concentrate	4.9	2.0	shift in 7.1 peak
		$A_{9.1}/A_{7.0}$	$A_{6.2}/A_{7.0}$	
3	Scour-1, feed	1.5	.84	
4	Scour-1, PEA permeate	2.6	.72	
5	Scour-1, CA permeate	(essentially	KBr spectrum)	
6	Scour-1, concentrate	1.7	.81	
		$A_{9.1}/A_{7.0}$	$A_{6.2}/A_{7.0}$	
7	Scour-2, feed	1.5	.71	
8	Scour-2, DM permeate	.8	.4	
9	Scour-2, concentrate	1.4	1.2	shift in 7.0 peak

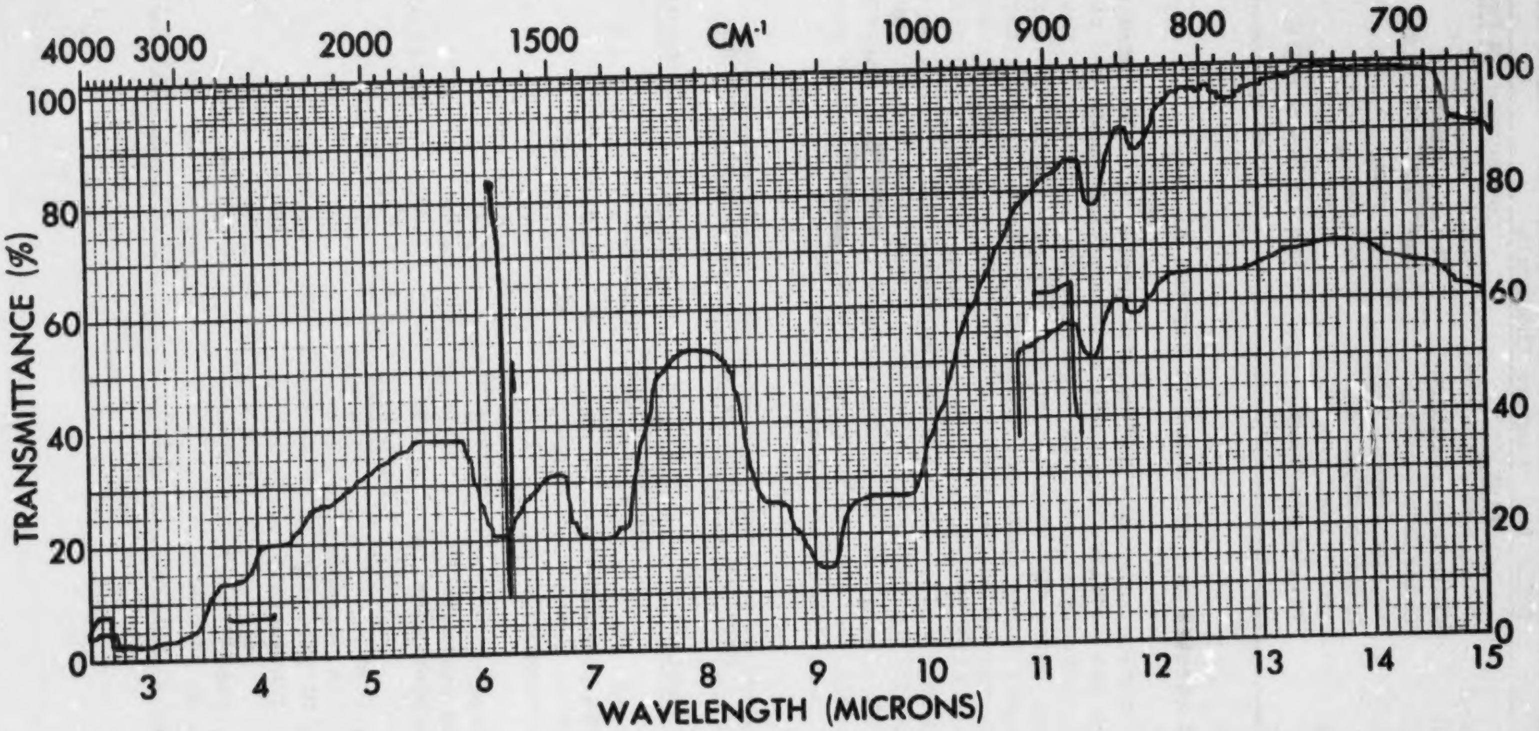
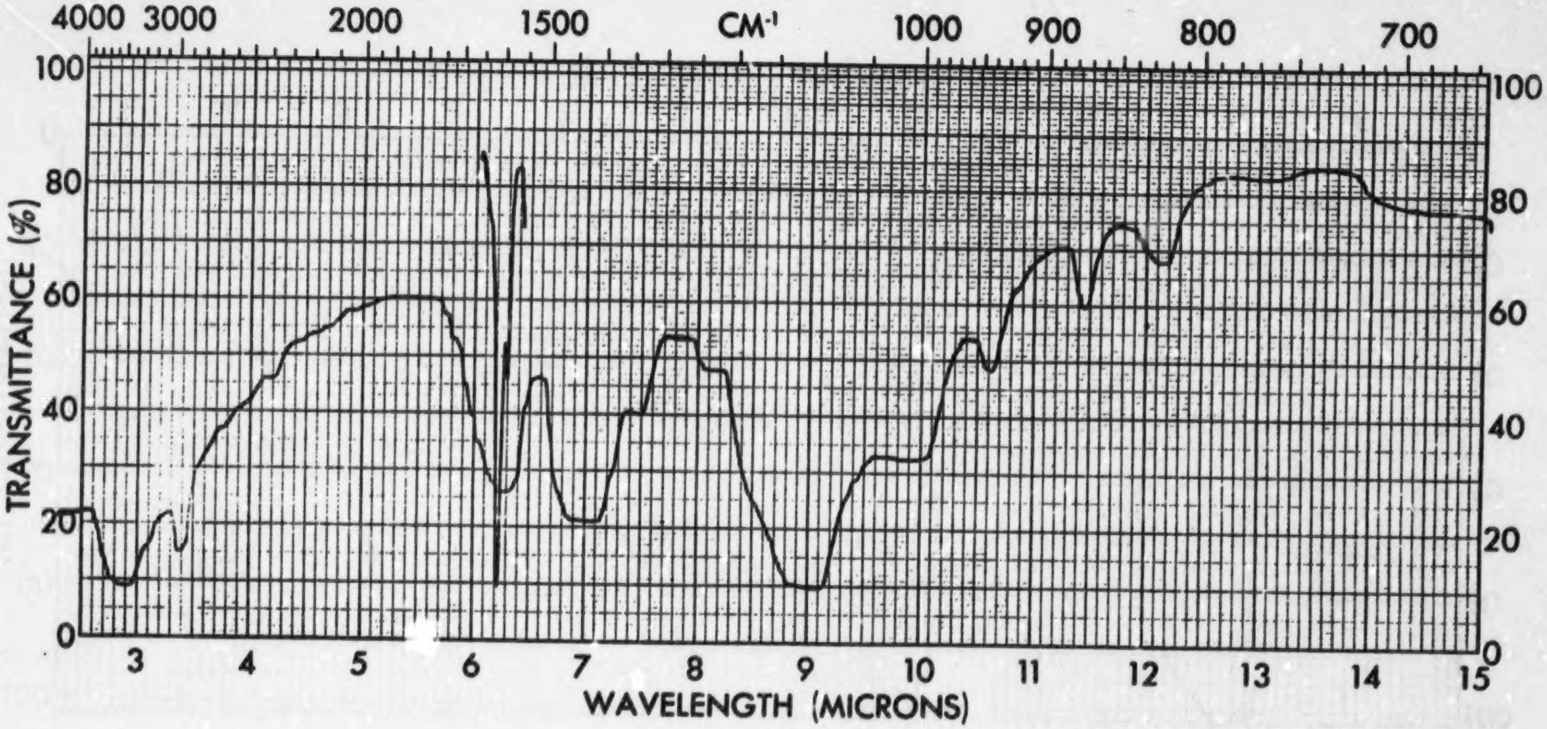


FIGURE A1 CTHF-2 RESIDUE



. FIGURE A2 SCOUR-1, FEED RESIDUE, CTHF-3

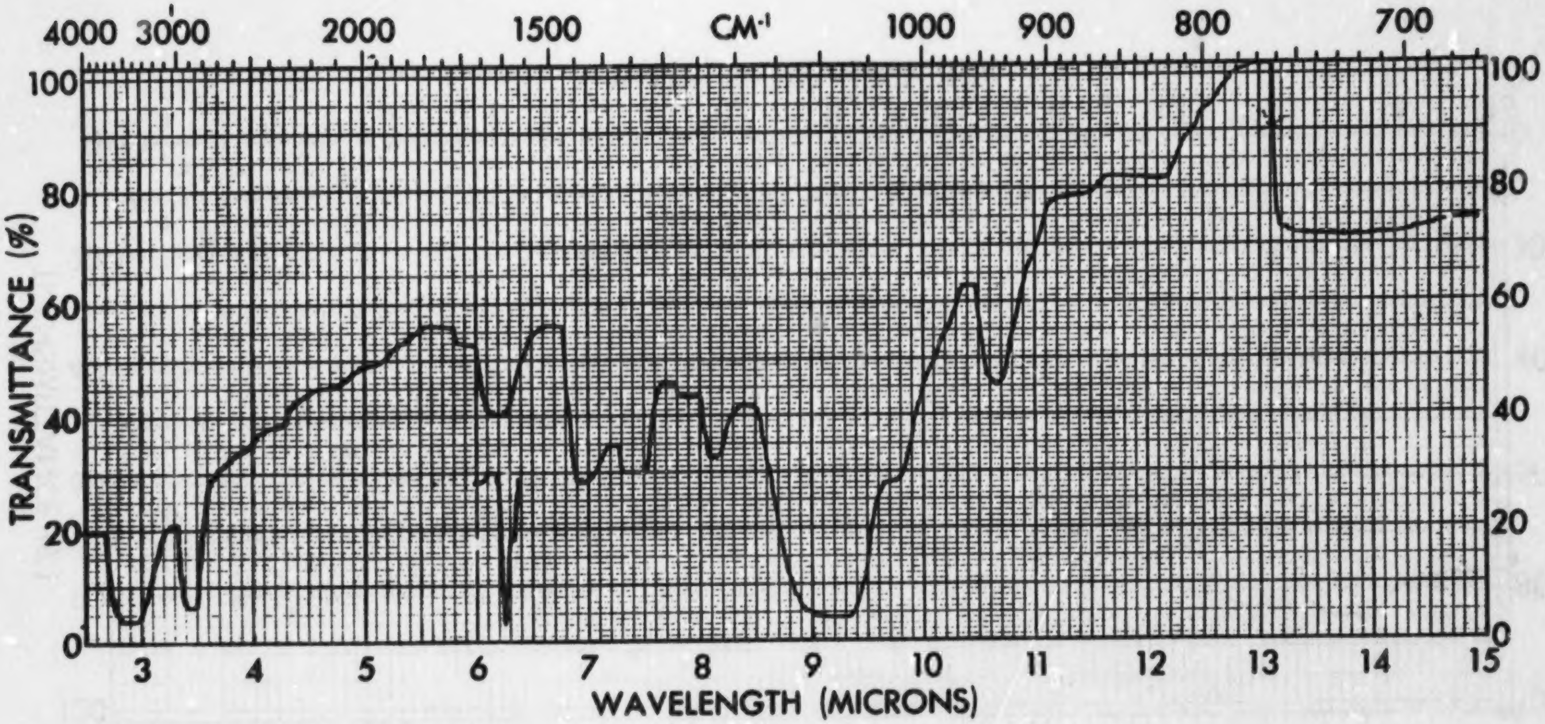


FIGURE A.3 SCOUR-1, PEA PERMEATE RESIDUE, CTHF-4

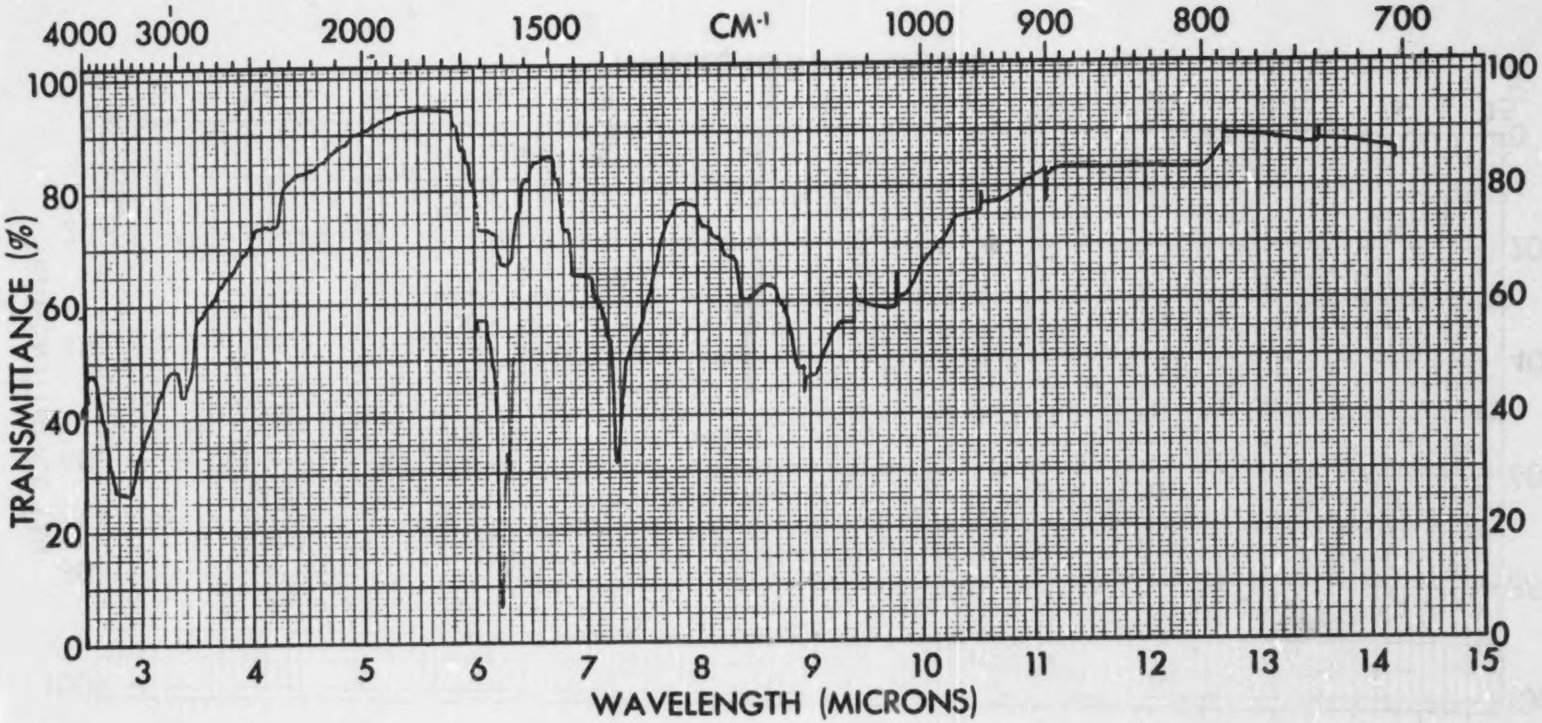


FIGURE A4 SCOUR-1, CA PERMEATE RESIDUE, CTHF-5

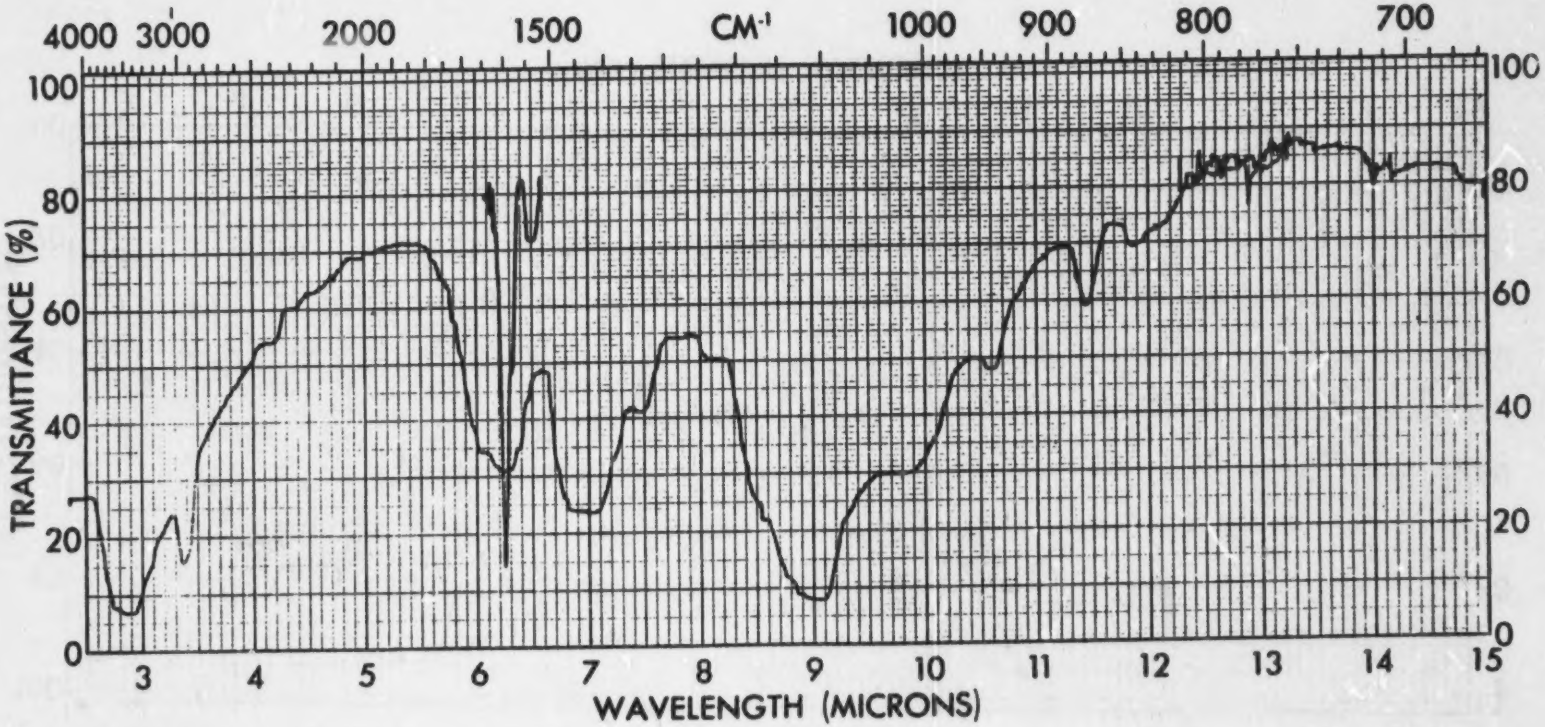


FIGURE A5 SCOUR-1, CONCENTRATE RESIDUE, CTHF-6

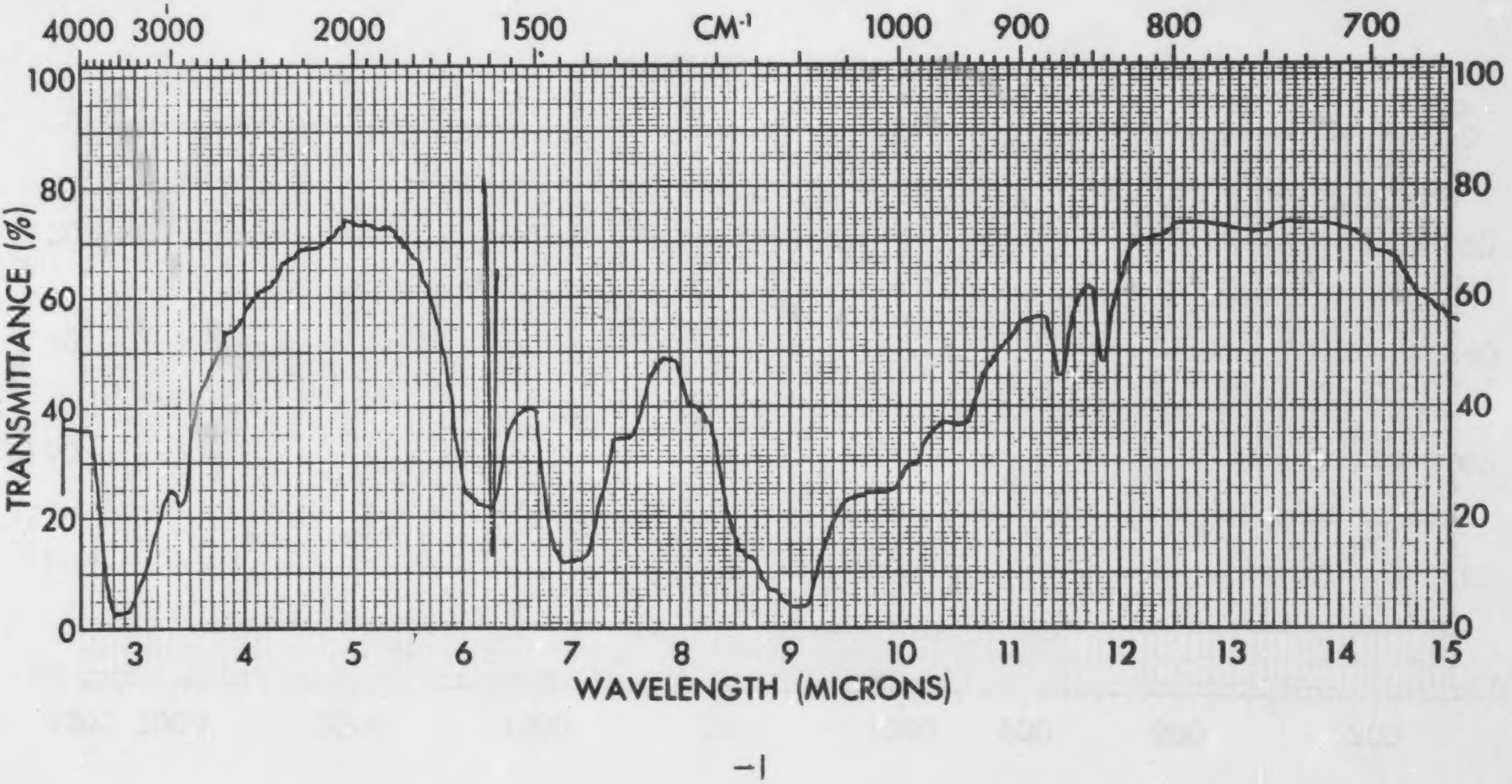


FIGURE A6 SCOUR-2, FEED RESIDUE, CTHF-7

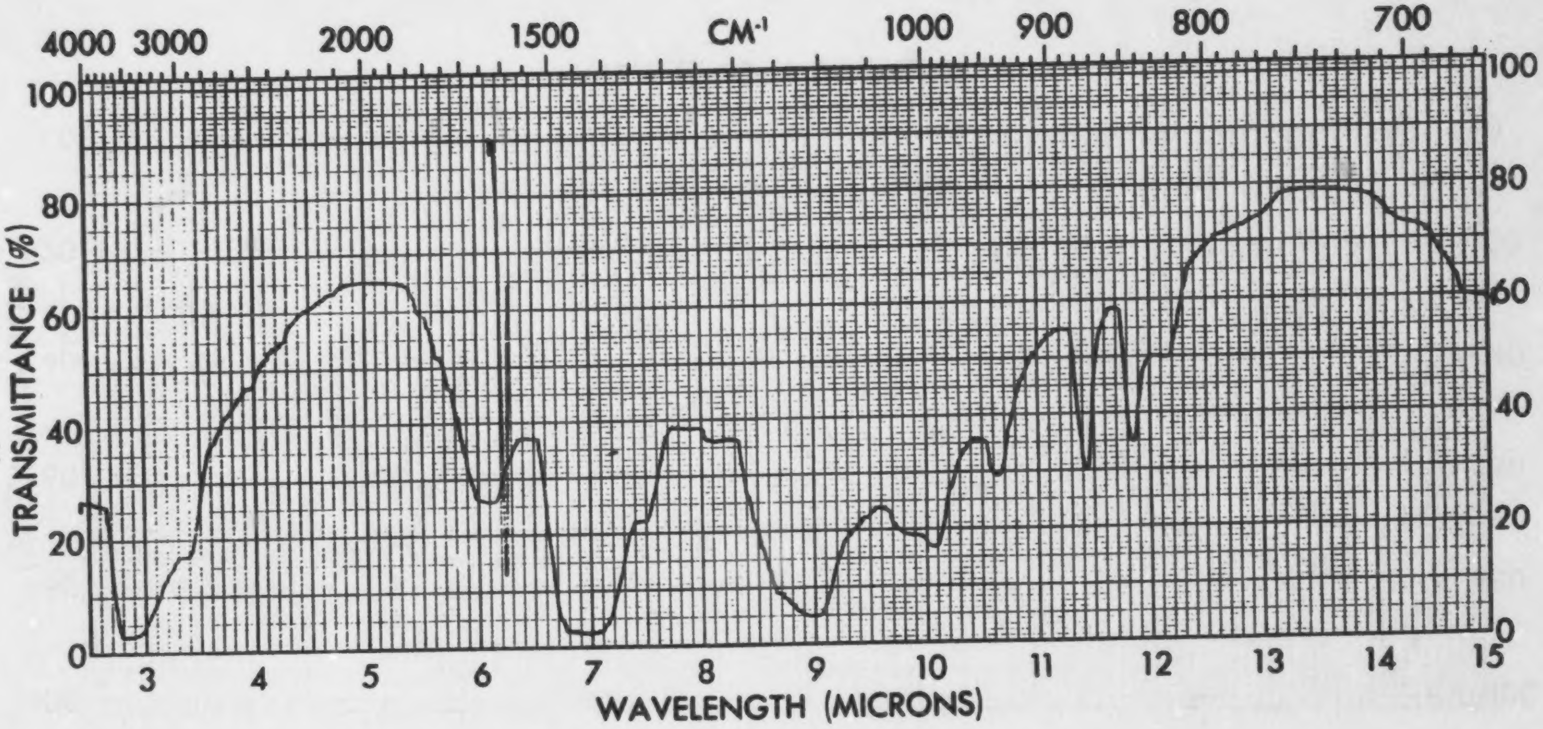


FIGURE A7 SCOUR-2, DM PERMEATE, CTHF-8

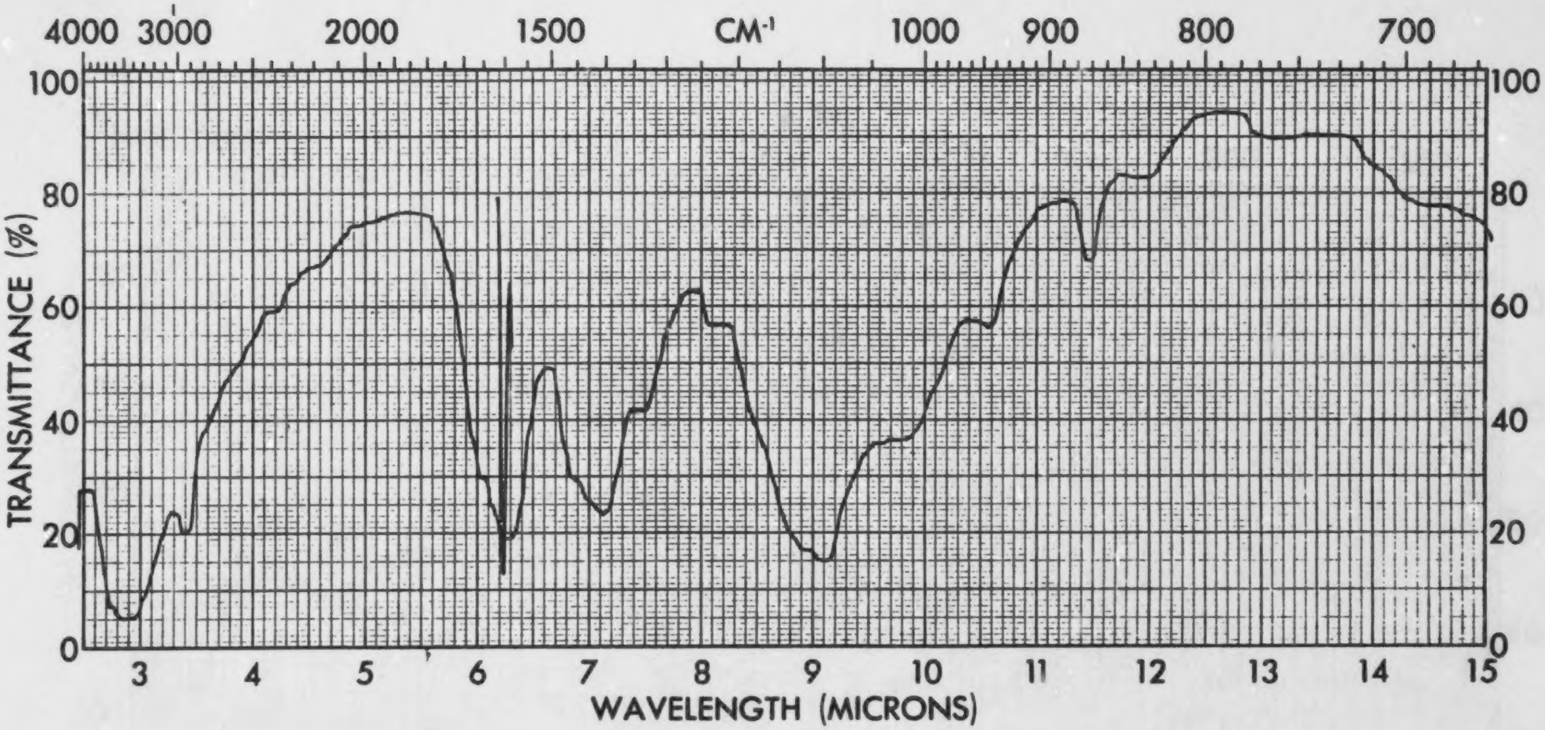


FIGURE A8 SCOUR-2, CONCENTRATE, CTHF-9

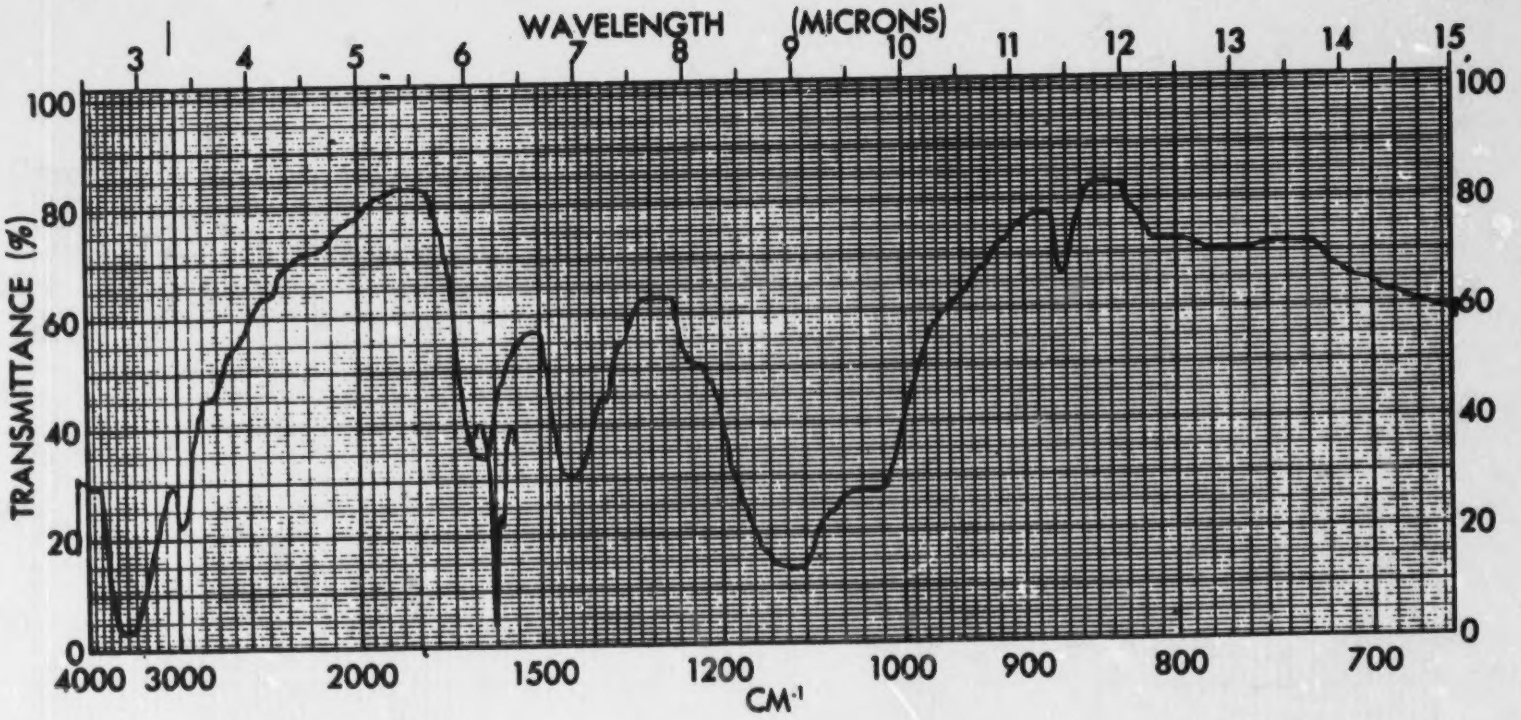


FIGURE A9 DYE-1, FEED, CTHF-10

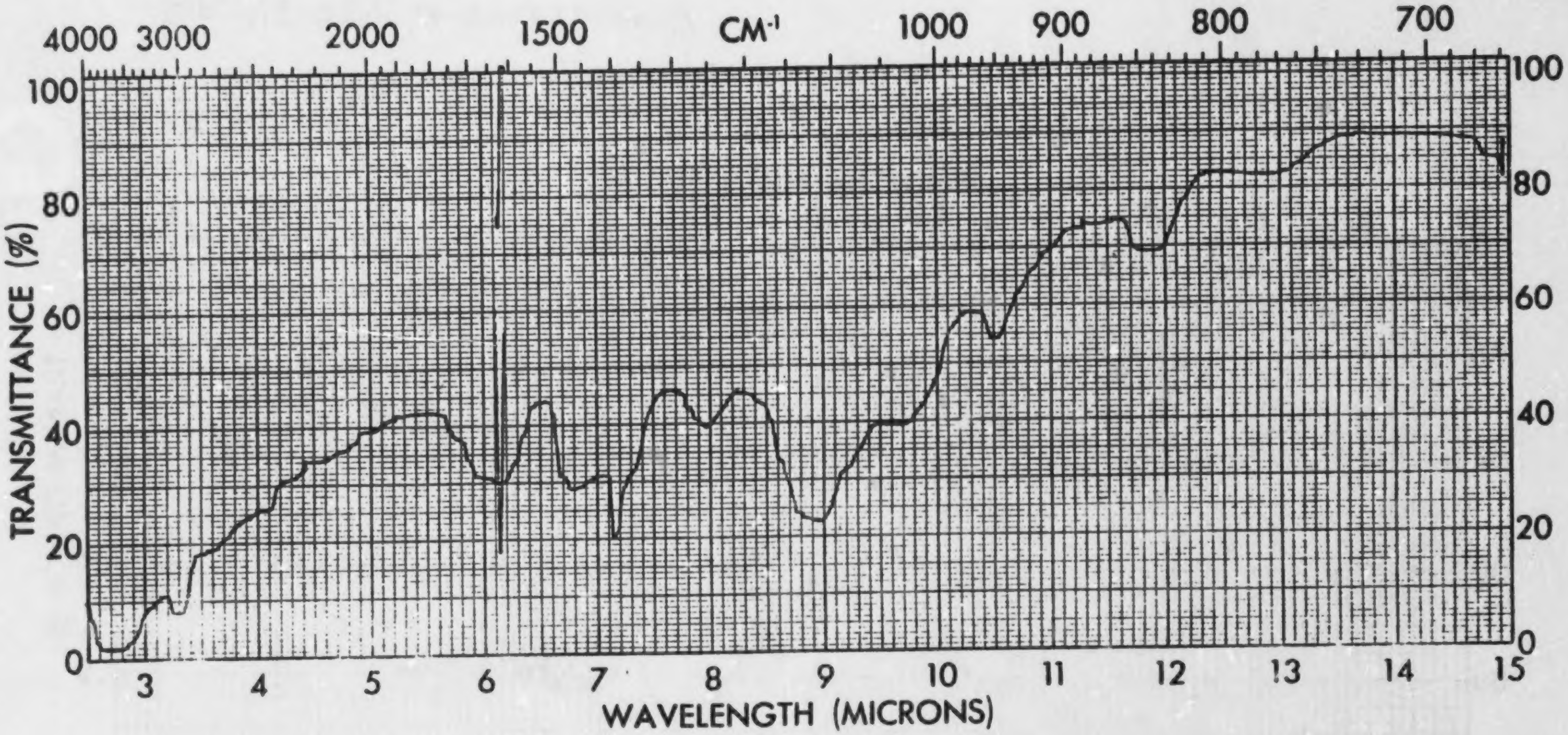


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FIGURE A10 DYE-1, PEA PERMEATE, CTHF-11

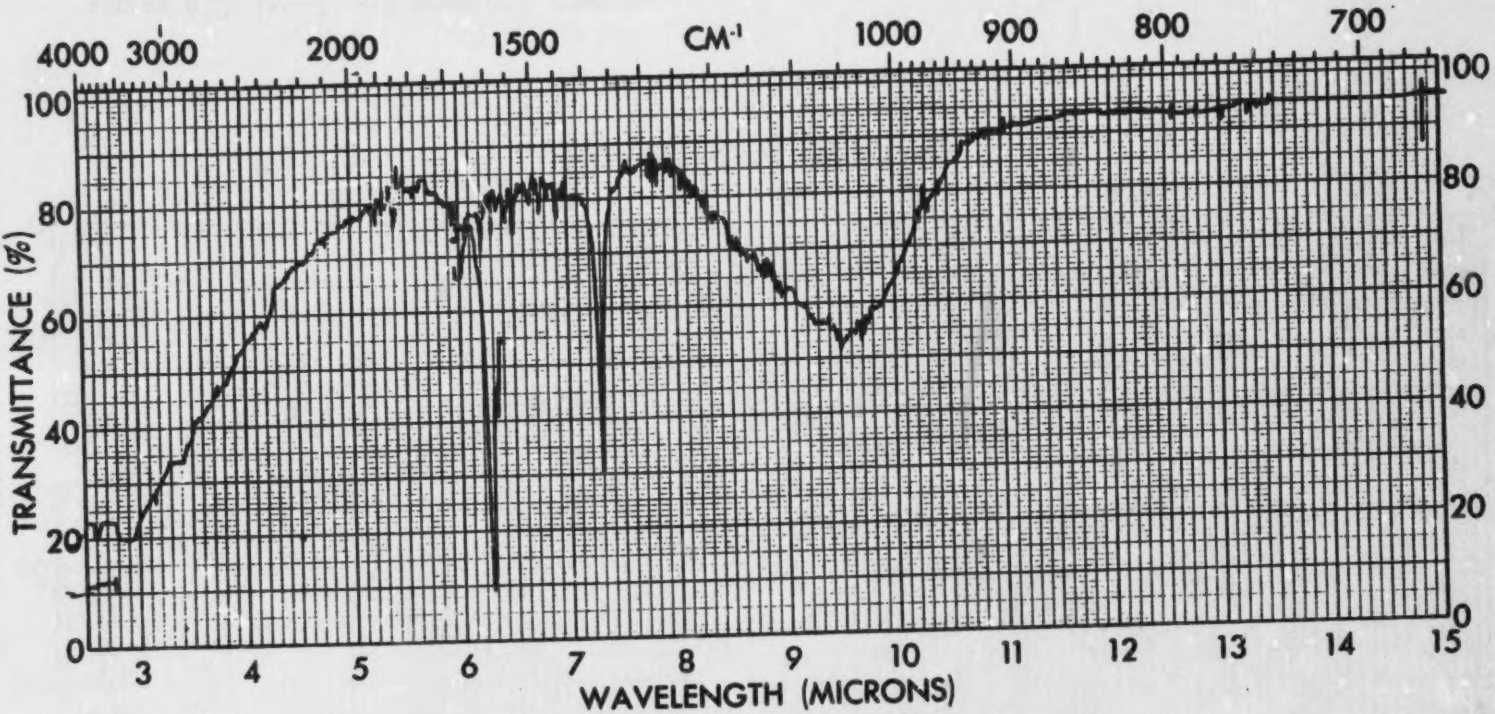

FIGURE A11 DYE-1, CA PERMEATE, CTHF-12

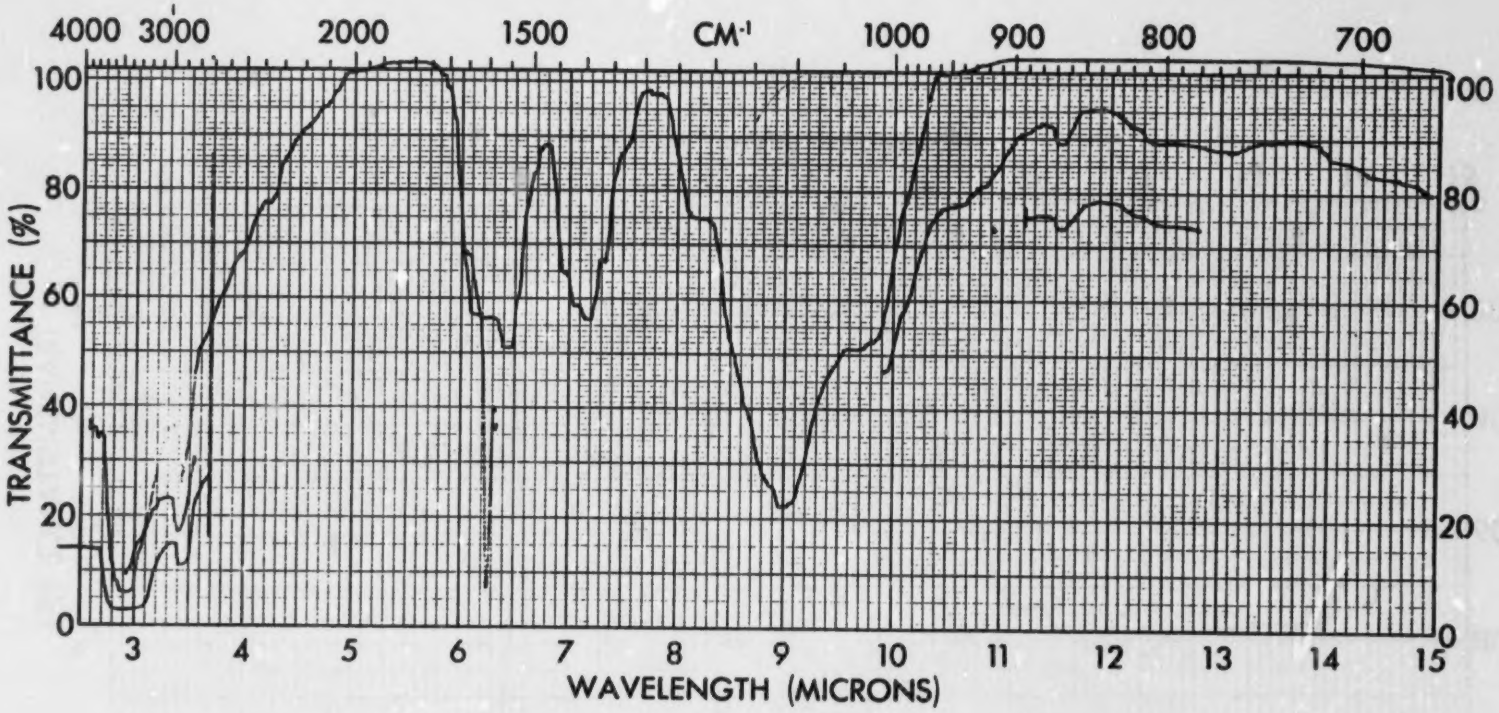

FIGURE A12 DYE-1, CONCENTRATE, CTHF-13

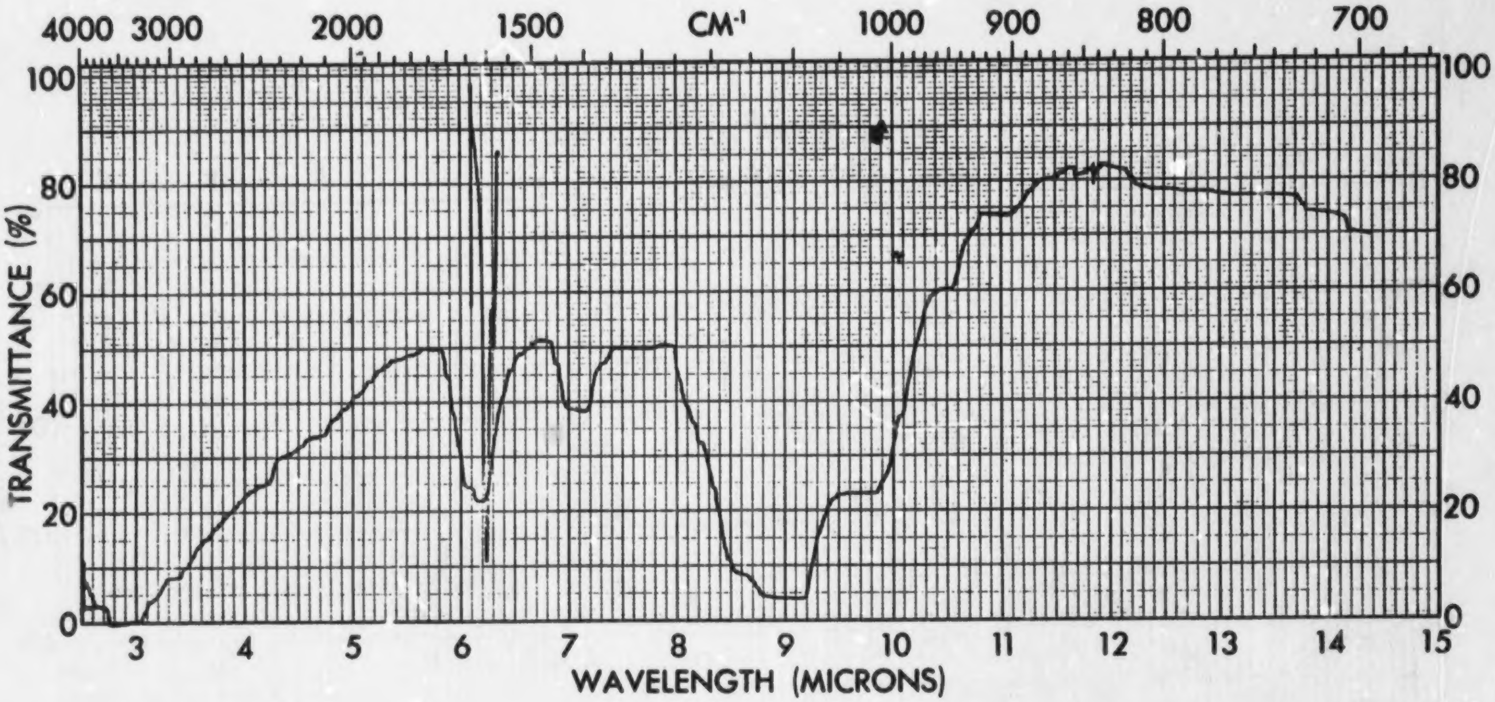

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FIGURE A13 DYE-2, FEED, CTHF-14

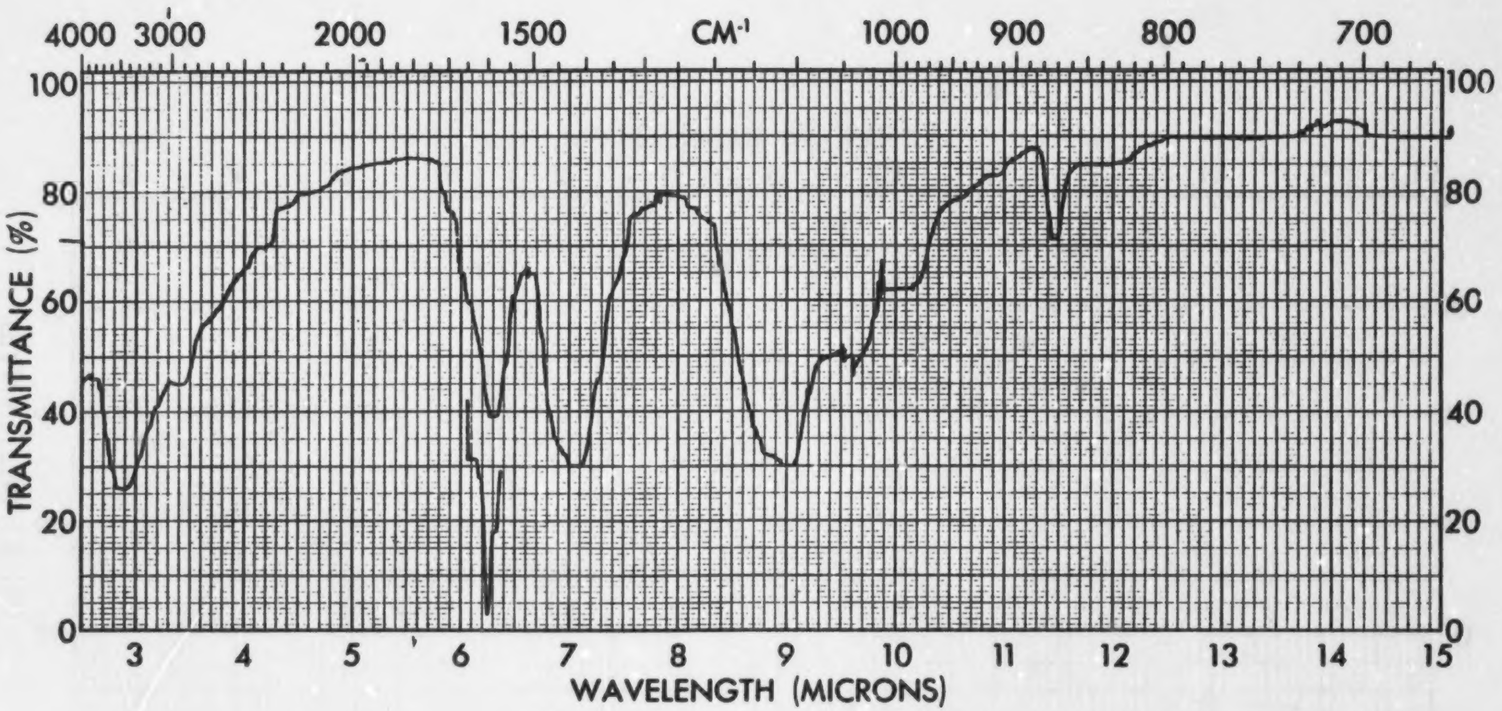


FIGURE A14 DYE-2, DM PERMEATE, CTHF-15

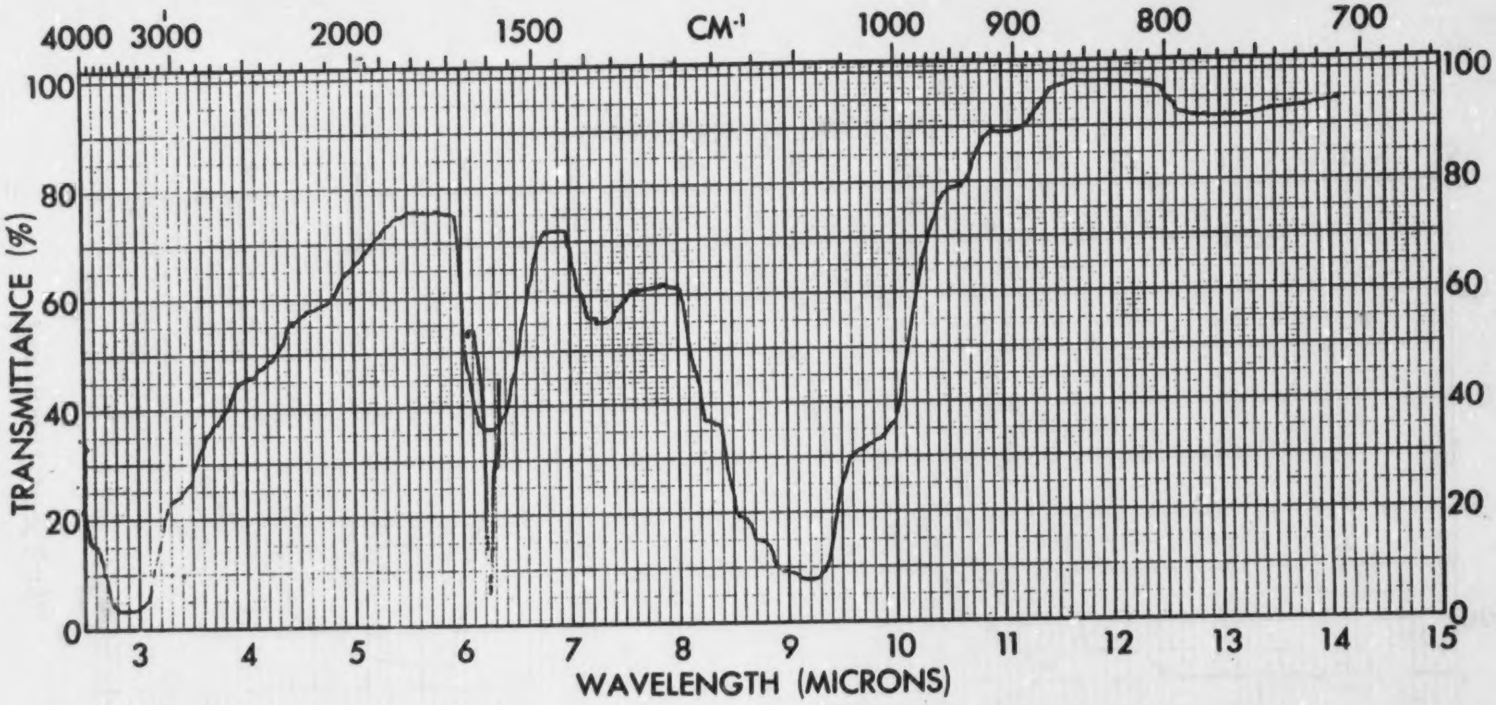


FIGURE A15 DYE-2, CONCENTRATE, CTHF-16

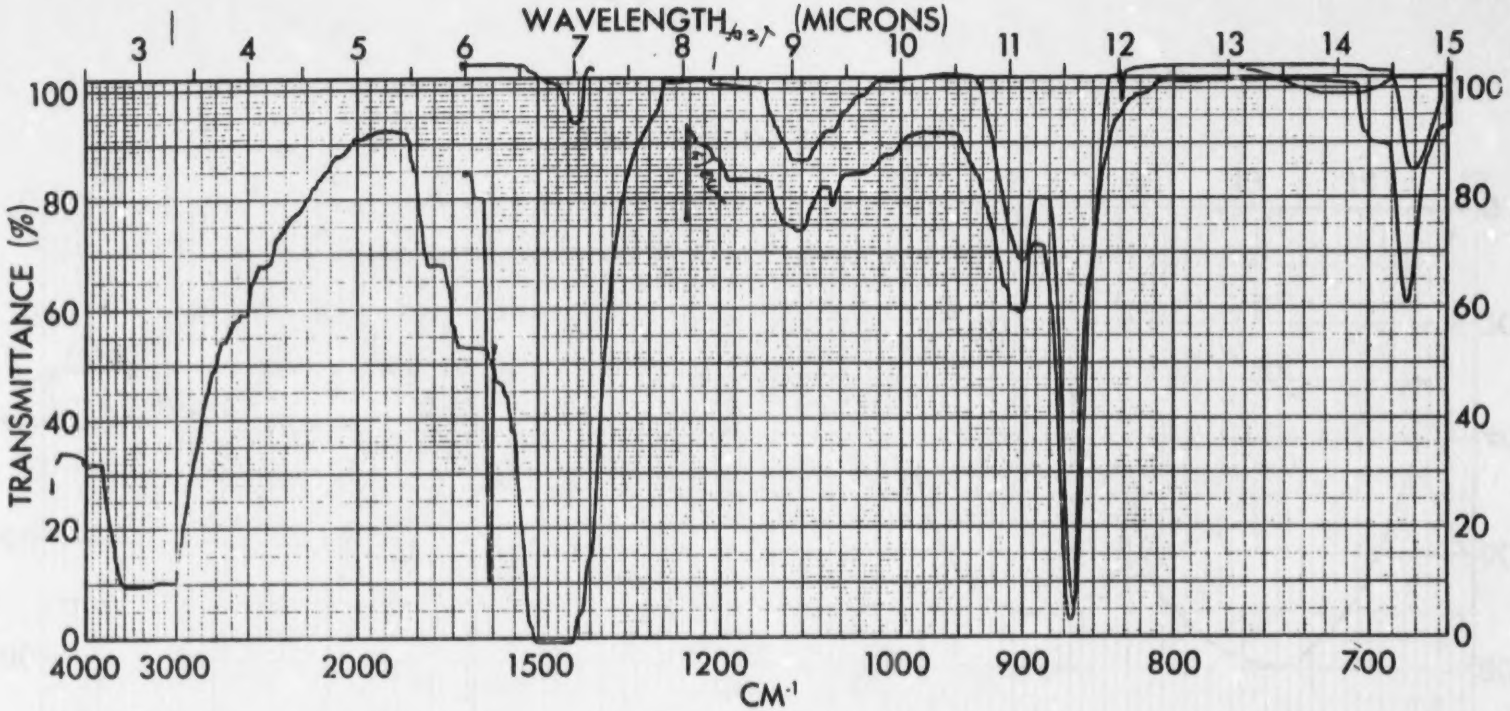


FIGURE A16 Na_2CO_3 RESIDUE

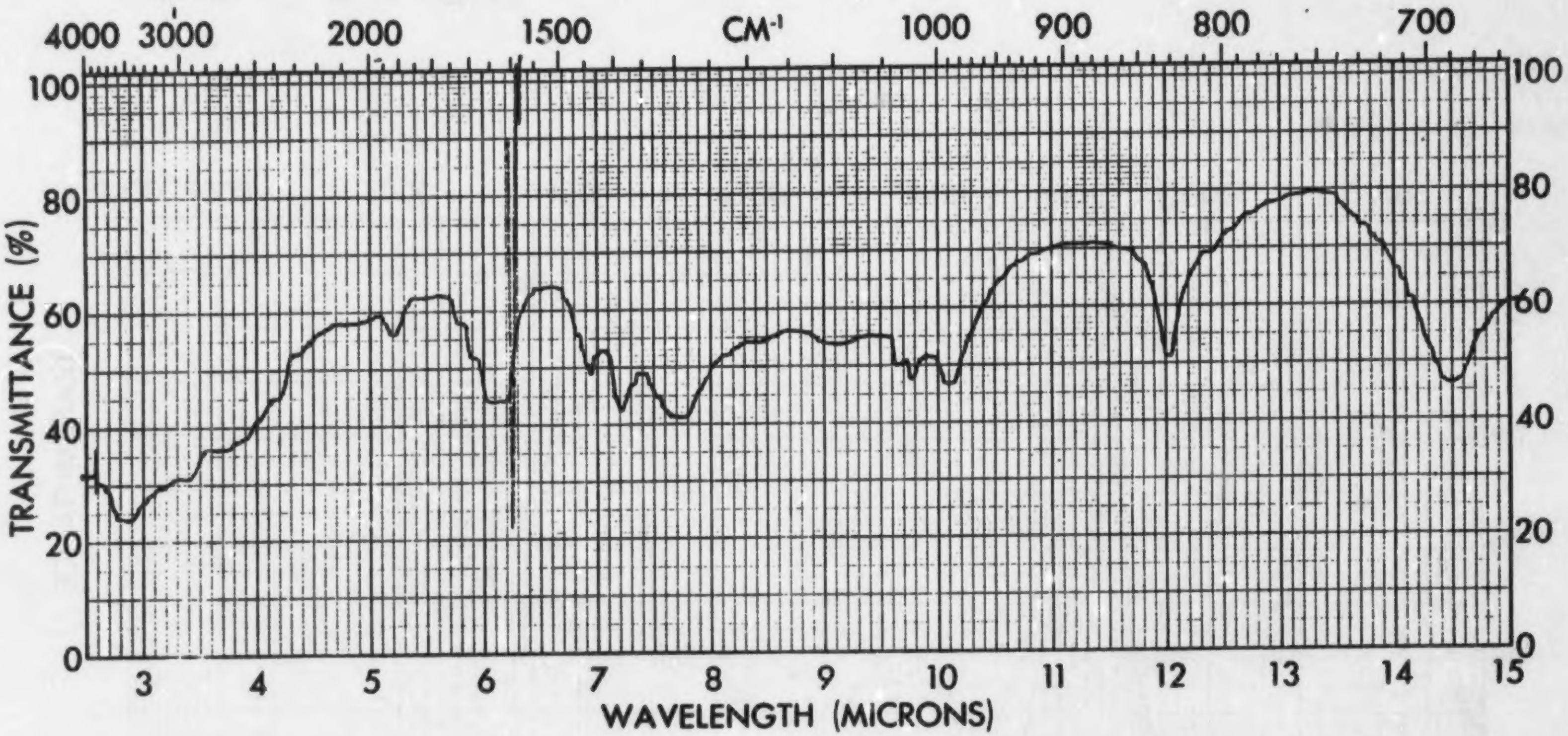


FIGURE A17 $NaHCO_3$ POWDER

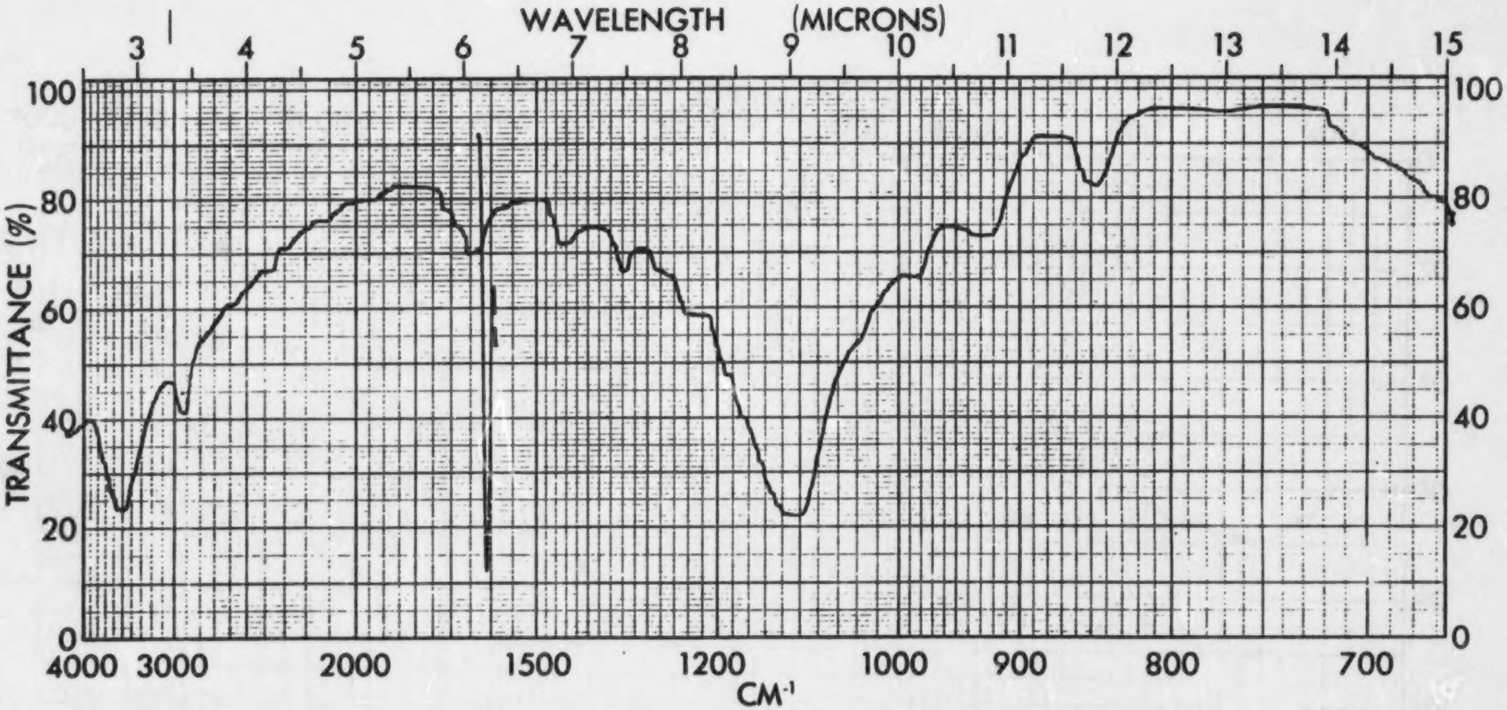


FIGURE A18 SIZE, POWDER

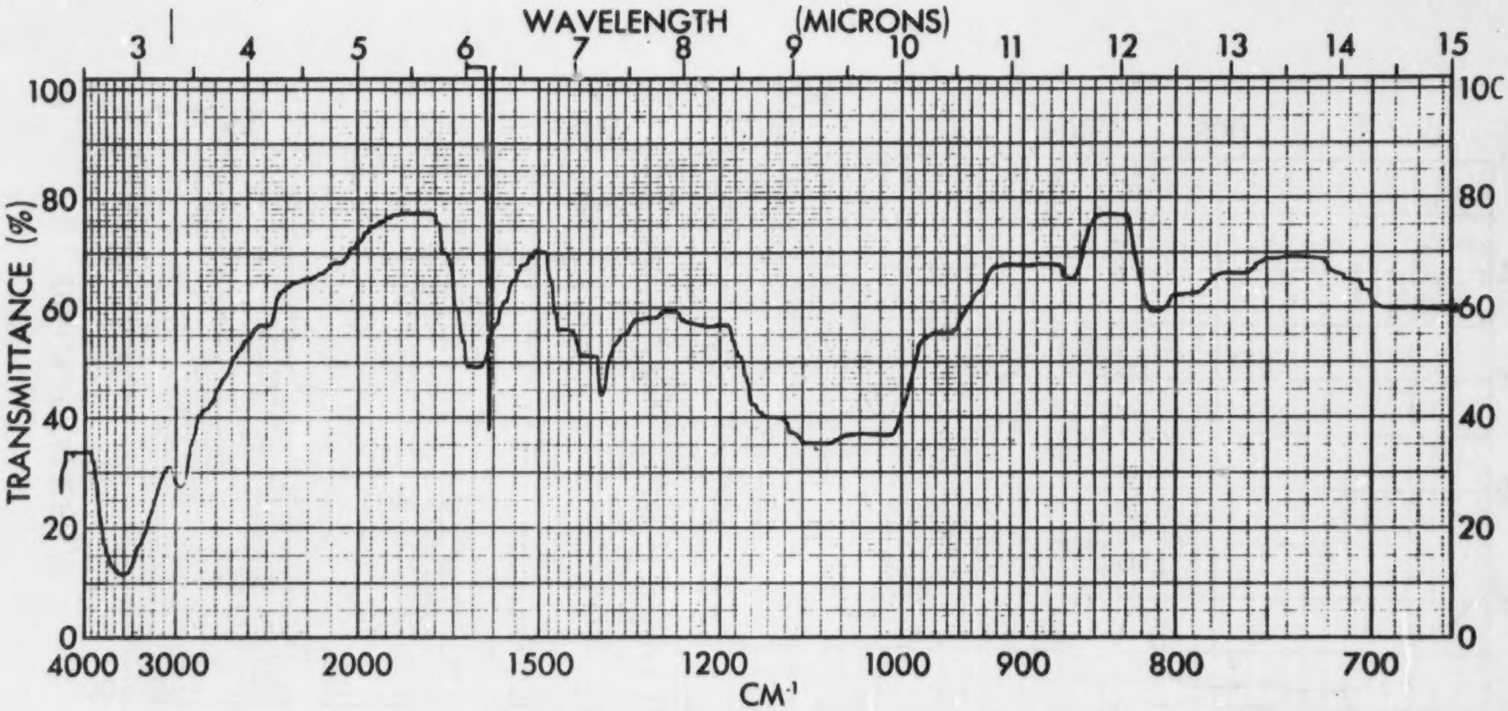


FIGURE A19 THICKNER, POWDER

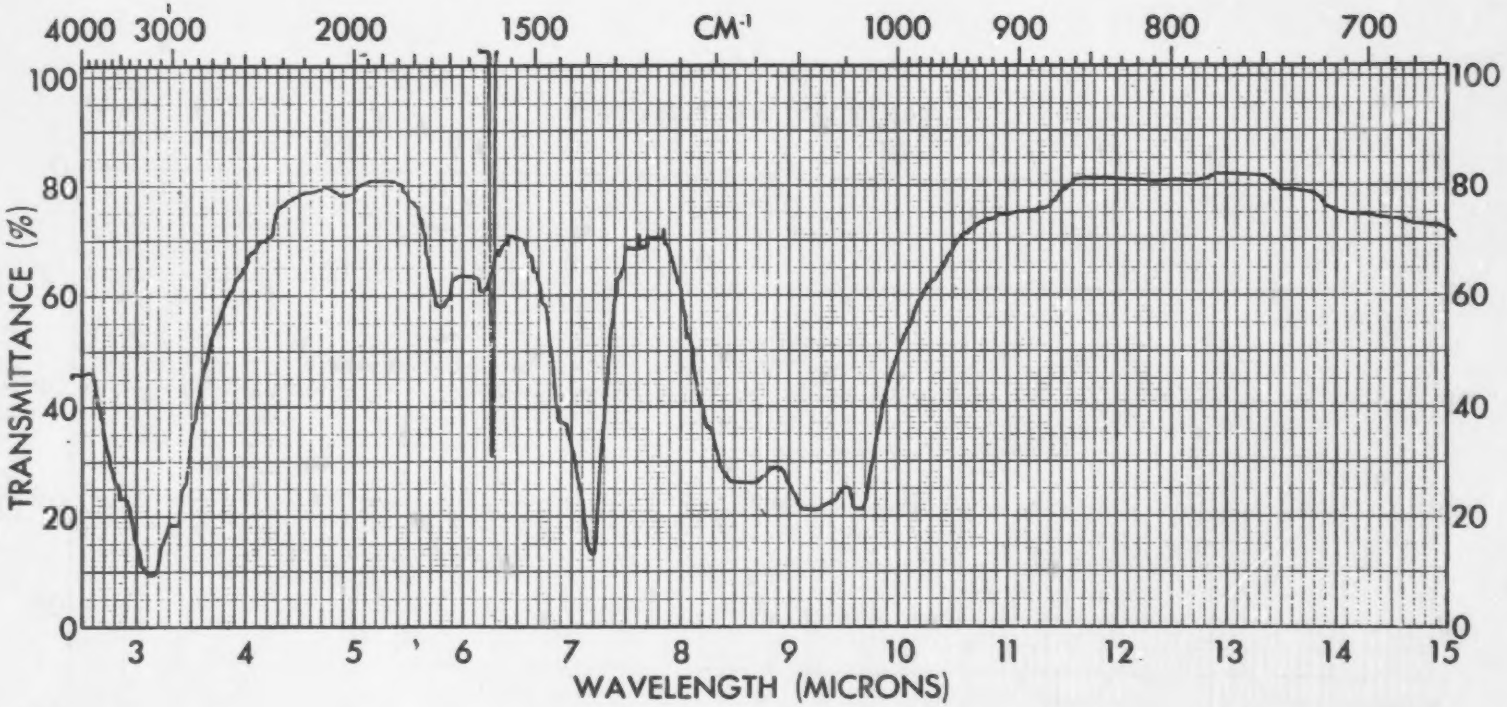


FIGURE A20 DISPERSING-WETTING AGENT-1, RESIDUE

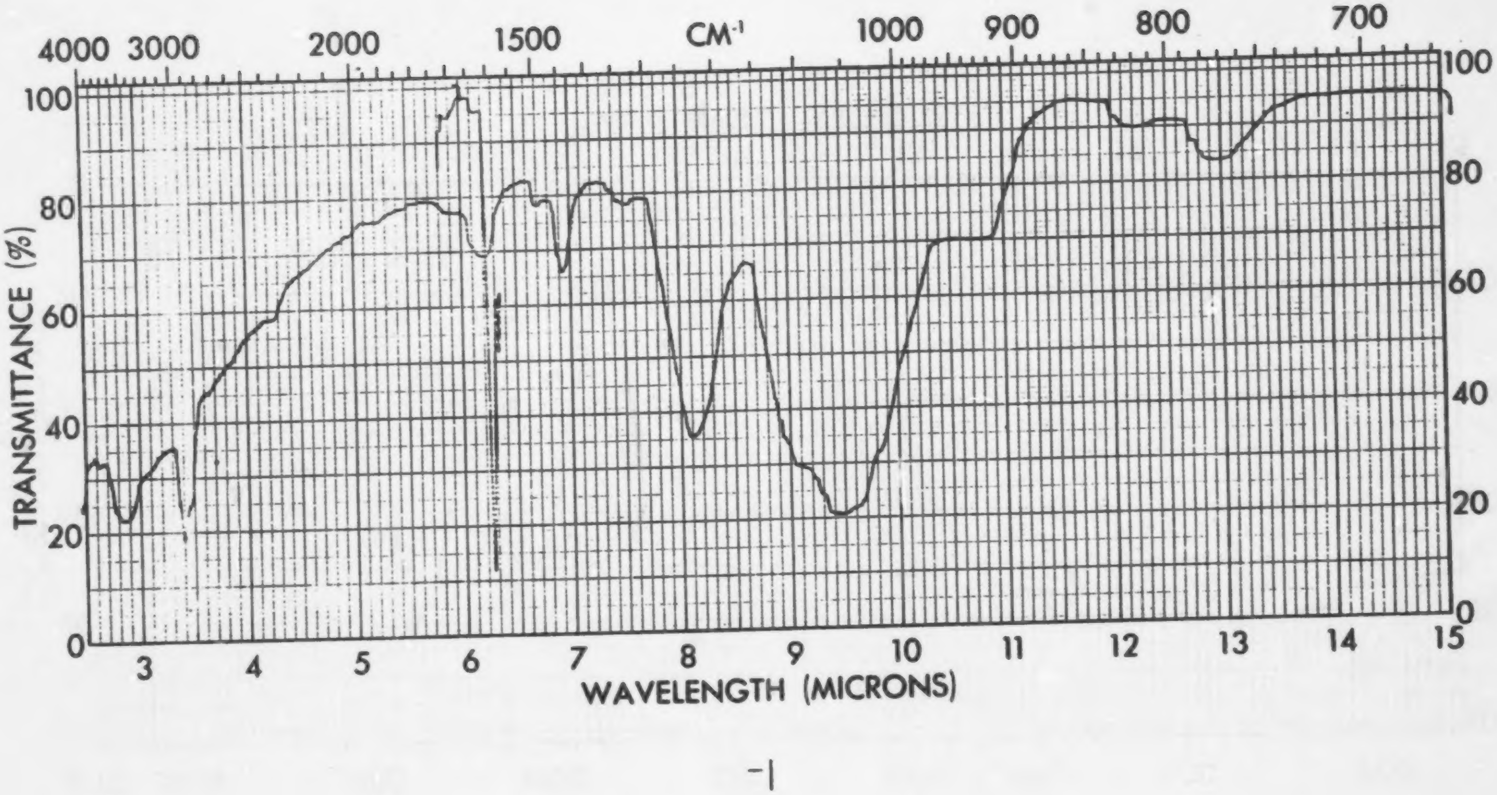


FIGURE A21 DISPERSING-WETTING AGENT-2, RESIDUE

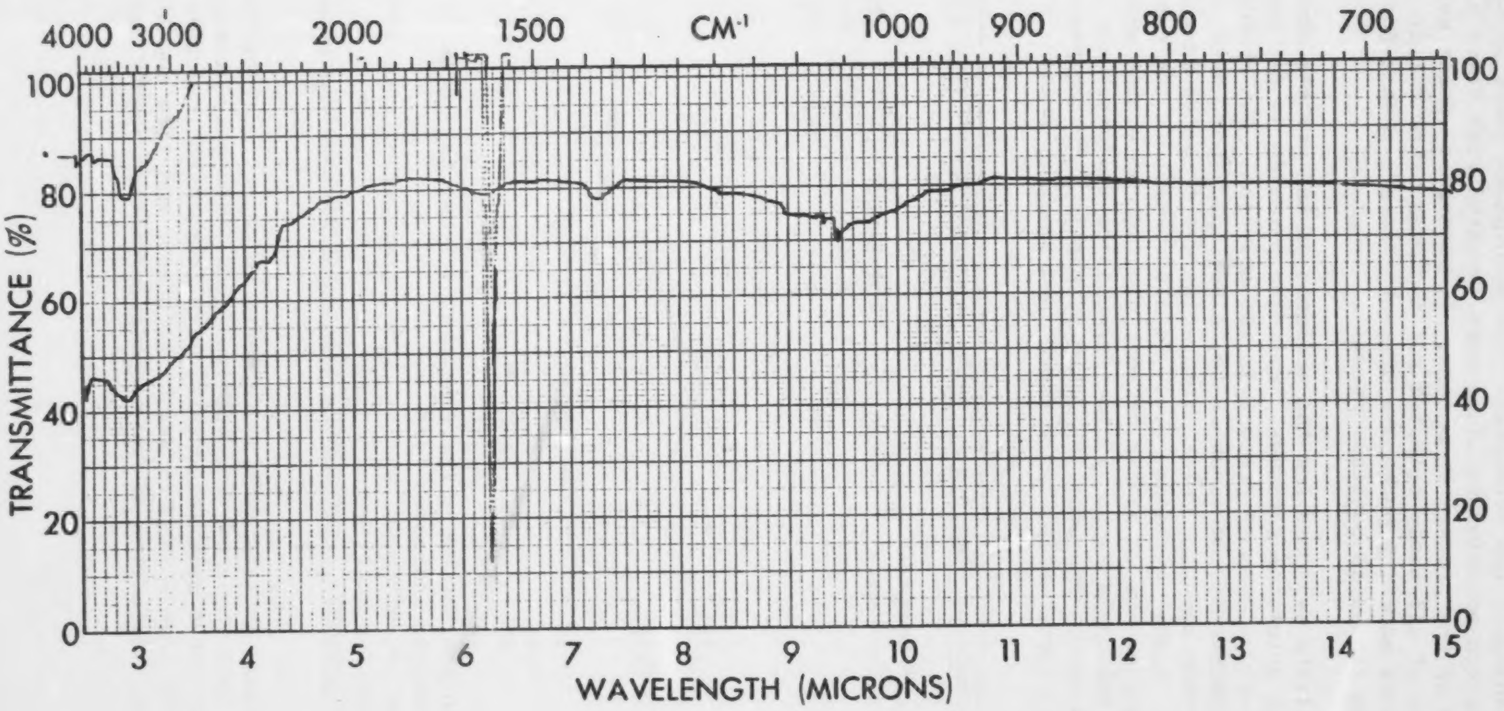


FIGURE A22 KBr PELLET

A second analysis was attempted. The absorption peaks in the spectra of the hyperfiltration solution residues were compared with three selected peaks in the spectra of the process chemicals. If absorption peaks were found to match the peaks of all those selected for a process chemical it is listed as present. If more matching peaks are present than are absent for a process chemical, it is listed as possibly present.

Table A4 lists evidence for the presence of the process chemicals in the hyperfiltration solutions residues. Hydrogen peroxide is not expected to occur in the residue even if present in the solution. The chemicals evaluated are carbonate and bicarbonate, size, thickener, dyes, and two dispersing/wetting agents. Color, indicating dye presence in residue, is denoted by C. Visual evidence, e.g., film formation in the residue indicating the presence of either or both the high molecular weight size and thickener, is indicated by R. If the formulation indicates the chemical's presence, F is used. Presence indicated by the ir matching-peaks analysis is ir and (ir), representing the presence and possibly present categories.

TABLE A4. Presence of Components in the Hyperfiltration Solution Residues

CTHF No.	Components					
	Carbonate/ Bicarbonate	Size	Thickener	DW-1	DW-2	Dyes
10			F,ir	F,(ir)	F,ir	F,C
11			(ir)	(ir)	ir	
12			*			
13		R	R,ir	(ir)	ir	C
14		R	F,ir,R	F	F	F,C
15	(ir)			(ir)		
16		R	R,ir			C
3	F,ir		(ir)		F,ir	
4					ir	
5			*			
6	ir	R,(ir)	R,(ir)		(ir)	
7	F,ir	ir	(ir)		F,(ir)	
8	ir	ir				
9	ir	R,(ir)	R,ir			

*A component in the KBr has a sharp peak at 7.3 μm, this peak is observed.
C - color in residue indicates dyes.
R - observation in residue of a film, indicating presence of either size or thickener, or both.
F in both formulations, ir-presence, (ir)-presence possible.

The cellulose acetate membrane appears to reject most of the ir-active components of both the scour and the dye feeds. The poly(ether/amide) and the dynamic membranes show selectivity with respect to ir-active components. The dyes and the high molecular weight thickener and size appear to be highly rejected. Rejection of the dispersing/wetting agents may not be as effective.

APPENDIX B

Interpretation of Results

The experiment involved concentrating an initial volume while producing a permeate volume. Therefore, it is necessary to appropriately interpret the results to estimate the values of the rejection. In the following discussion M symbolizes the mass of fluid in the concentrate flow, $\dot{m}$ symbolizes a mass flow rate, C symbolizes the mass concentration of solute, t symbolizes time, and γ symbolizes the rejected fraction of solute. Only values for the mean value of rejection may be calculated. Subscripts are used as follows: "c" pertains to the concentrate, "f" to the feed, "p" to the permeate, "e" to evaporation, and "l" to leak.

The initial mass, M_f, is depleted in general by evaporation, leaks (if applicable), and permeation. The following equation is expected to apply for two membranes.

$$\frac{dM}{dt} = - \dot{m}_e - \dot{m}_l - \dot{m}_{p1} - \dot{m}_{p2} \qquad \text{(B1)}$$

Integration yields an expression for the mass at any time

$$M(t) = M_f - {}_0\int^t (\dot{m}_e + \dot{m}_l + \dot{m}_{p1} + \dot{m}_{p2})dt \qquad \text{(B2)}$$

When t becomes the elapsed time for the experiment, the corresponding M value becomes M_c, the concentrate mass. Separate observations of M(t), measured as fluid depth during the experiment, allow an estimate of the value of m_e (evaporation rate). The absolute measurement of M_f and other values is uncertain due to ignorance of the volume of pumps, fittings, modules. Corrections may be applied to promote the integrity of the volume estimate based on relative values of concentrate and feed data provided by analysis.

The volumes recorded during operation of the test procedure are shown in Table B1. The initial volumes and concentrate volumes were obtained by measuring the level in the tank (top of tank to fluid level). To the value thus obtained was added 20 dm^3 to account for the internal volume of pipes, etc. The permeate volumes were obtained by measurement of fluid level in the containers and by integration of the permeate rates observed. The leak in Run 2 was measured in terms of its duration and rate. The vaporized volume is simply the volume required to close the fluid balance. Runs 1 and 3 show the permeate volume as the sum of two numbers which are, respectively, the PEA permeate and CA permeate. All values in Table B1 are subject to errors in observation through at least the following mechanisms: (1) Poor approximation in system hold up volume, (2) tanks not exactly level, (3) ordinary measurement of length uncertainty, and (4) difficulty with foaming fluid level sensing. Therefore the use of total solids measurements to improve the volume estimates has been employed. The following describes the methodology of calculating the values of solute in the leak fluid and the determination of the permeate volume fraction from concentration data. An equation for the concentration of a particular solute may be written based on a differential mass balance, using γ to symbolize rejection:

$$\frac{d(MC)}{dt} = -\dot{m}_1 C - \dot{m}_{p1}\,(1 - \gamma_1)C - \dot{m}_{p2}\,(1 - \gamma_2)C \qquad \text{(B3)}$$

Evaporation has been deleted from this equation by assuming that the solute is non-volatile. Expanding d(MC) to CdM + MdC and substitution from equation (B1) for dM/dt gives

$$M\frac{dC}{dt} = \gamma_1\,\dot{m}_{p1}\,C + \gamma_2\dot{m}_{p2}\,C + \dot{m}_e C.$$

TABLE B1. Recorded Volumes

	Run 1	Run 2	Run 3	Run 4
Initial Volume (dm^3)	593	371	429	465
Concentrate Volume (dm^3)	90	60	73	65
Permeate Volume (dm^3)	282 + 221	214	188 + 168	300
Leak Volume (dm^3)	0	29	0	0
Vaporized Volume (dm^3)	0	68	0	100
Recovery = $\frac{\text{feed-concentrate}}{\text{feed}}$	0.848	0.838	0.824	0.860

Division by MC renders the variables separated if γ is independent of C

$$\frac{dC}{C} = \frac{\gamma_1\dot{m}_{p1} + \gamma_2\dot{m}_{p2} + \dot{m}_e}{M}\,dt \qquad \text{(B4)}$$

Substitution of equation (B2) for M allows straightforward integration, which must be done numerically except for special cases. One important special case has no leak ($\dot{m}_1 = 0$), a neglibible evaporation rate, and constant permeate rates. Equation (B4) after substitution of equation (B2) for M yields

$$\ln\left(\frac{C_c}{C_f}\right) = \int_0^t \frac{\gamma_1\dot{m}_{p1} + \gamma_2\dot{m}_{p2}}{M_f - \dot{m}_{p1}\,t - \dot{m}_{p2}t}\,dt = \left\{\frac{\gamma_1\dot{m}_{p_2} + \gamma_1\dot{m}_{p2}}{\dot{m}_{p1} + \dot{m}_{p2}}\right\}\ln\left\{\frac{M_f}{M_f - M_1 - M_2}\right\}$$

$$\frac{C_c}{C_f} = \left\{\frac{M_f}{M_f - M_1 - M_2}\right\}^{\frac{\gamma_1 \dot{m}_{p1} + \gamma_2 \dot{m}_{p2}}{\dot{m}_{p1} + \dot{m}_{p2}}} \tag{B5}$$

In equation (B5) M_1 and M_2 are the permeate total mass associated with membranes 1 and 2, respectively. Equation (B5) holds for one membrane as well and is also valid for non-constant flow rates if the rates are proportional.

A global mass balance equation may be written as

$$M_f C_f = M_c C_c + M_1 \overline{C}_{p1} + M_2 \overline{C}_{p2} \tag{B6}$$

The overbar designates mixed average permeate. Division by C_f, substitution of equation (B5) for C_c/C_f and substitution of $M_c = M_f - M_1 - M_2$ leads to

$$R_1 \frac{C_{p1}}{C_f} + R_2 \frac{C_{p2}}{C_f} = 1 - (1-R)^{\frac{R_1(1 - \gamma_1) + R_2(1 - \gamma_2)}{R}} \tag{B7}$$

where $R_1 = M_1/M_f$, $R_2 = M_2/M_f$, and $R = R_1 + R_2$. R is commonly called the recovery.

Equation (B5) may be used to calculate the average rejection of the two membranes based on the recovery and chemical analysis of concentrate and feed. Equation (B7) may be used to calculate the rejection based on analysis of permeate and feed. An auxiliary equation permits the calculation of

$$\frac{1 - \gamma_1}{1 - \gamma_2} = \frac{C_{p1}}{C_{p2}} \tag{B8}$$

rejection for either membrane by itself. Ideally at the recovery value observed the solute mass balance (B6) is satisfied and the calculated rejections from (B5) and (B7) are identical. This never happens precisely due to experimental uncertainty. In the interpretation herein calculations of mass balance in terms of ratio of the right side to left side of equation (B6), rejection based on permeate and feed data have been made. These calculations have been made at various levels of recovery near the actual recovery noted in testing. The results for total solids determination have been employed for this exercise. Table B2 shows a typical result for the fourth run (scour fluid with dynamic membrane). Using volume observations the best estimate of recovery (vapor + permeate)/feed was 0.86 while the recovery which yields essentially unity for the mass balance ratio (right side divided by left side of equation (B6)) was 0.82 (less than 4% different). In this case the use of the best mass balance yields good agreement between the rejection estimates and the recovery of 0.82 is adopted as the best estimate of actual volume recovery. The values of rejection calculated from permeate and feed data differ by less than 1 percent, while the rejection calculated using concentrate and feed data differ by over 10 percent. When differences occur in the rejection estimated from concentrate and feed compared to the rejection estimated from permeate and feed, the value of rejection should be estimated on the basis of permeate and feed data due to the reduced sensitivity to experimental uncertainty.

TABLE B2. Effect of Recovery on Mass Balance and Rejection

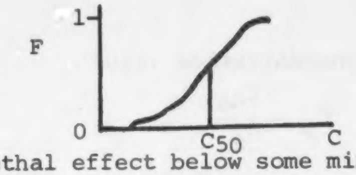
	Based on Volumes	Recovery with Best Mass Balance
Recovery	0.860	0.820
Mass Balance Ratio (final initial)	0.862	1.0
Rejection (using concentrate)	0.783	0.866
Rejection (using permeate)	0.882	0.875

In each of the four runs a calculation similar to that described for run 4 was made. In Runs 1 and 3, an improved recovery estimate using total solids data was found to agree reasonably well with the preliminary volume estimates. In Run 2 the total solids data do not allow a reasonable change in the preliminary estimate and which forces a solute mass balance. The best interpretations for the exact recovery are presented in Table 2 of the main text of the report. As shown in the table, 26 percent of the initial mass in Run 2 could not be accounted for in the sum of permeate and concentrate. Recheck of solids analysis shows no change and the conductivity data tend to collaborate the preliminary volume estimates so that no substantial improvement of the recovery estimate can be made. Run 2 was complicated by a leak and evaporation which tend to increase the difficulty in interpretation.

A computer program was prepared for detailed analysis of the specific solute analytical data. Upon receipt of data and execution of the program it was apparent that such an exercise would not be meaningful. For example, in Tables 4 through 7 few results for mass balance ratio were near unity. Therefore for the metals, a simple rejection calculation was adopted based on use of permeate and feed data. The concentrate and feed comparison is much more sensitive; i.e., errors in analysis or recovery estimated are amplified in rejection estimate. Only in cases where the permeate and feed data may not yield clearly defined results due to low concentrations will the concentrate analysis be important. In such a case, the presence of concentrated material in the concentrate stream indicates rejection has occurred.

For interpretation of organic solute data, depletion in the permeate together with enrichment in the concentrate yield confidence in estimating a substantial rejection of solute. Many solutes have such results and others have conflicting indications. Those which conflict by having one indication of rejection and another of no or negative rejection will be in violation of the mass balance. The more likely erroneous datum may be selected from such logic. The relatively unsatisfying statements "probably rejected", "probably not rejected", or "mixed indications" are really all the information that can be gleaned.

Following are a group of comments pertinent to the interpretation of the bioassays. A single toxicant is presumed to have a lethal fraction versus concentration curve F(C) as illustrated in the sketch below.



The curve shows no lethal effect below some minimum concentration rising to complete mortality at a higher concentration. The value of $C = C_{50}$ will produce lethal effects in half the subjects. If two lethal species are present the lethal fraction F may be determined on the basis of the individual components. Let $F_1(C_1)$ be the lethal distribution curve for specie 1 and $F_2(C_2)$ that for specie 2. Those dying from exposure to specie 1 will be $F_1(C_1)$ of those not dying from specie 2. Those dying from specie 2 will be $F_2(C_2)$ of those not dying from specie 1. If P_1 and P_2 represent the fractions killed by specie 1 and 2 respectively,

$$P_1 = (1-P_2)F_1(C_1)$$
$$P_2 = (1-p_1)F_2(C_2)$$

without synergistic effects, the total fraction killed is the sum of these, or F is

$$F = \frac{F_1(C_1) - F_2(C_2)}{1 - F_2(C_2)} + \frac{F_2(C_2) - F_1(C_1)}{1 - F_1(C_1)} + \frac{[F_1(C_1) - F_2(C_2)]^2}{[1-F_2(C_2)][1-F_1(C_1)]}$$

If the relative amounts of specie 1 and 2 are the same, or if F_1 and F_2 are identical functions, then $F(C_1+C_2)$ will behave exactly as a single component. Similar results are expected from situations with three or more components. In many but not all hyperfiltration systems the rejections of individual toxicants will not be largely different from each other so that the relative concentrations of substances will be preserved. It is not unexpected then for mixtures of toxicants to behave as a single toxicant (even with synergistic effects).

The action of hyperfiltration on a single toxicant is expected to produce a dilute and concentrated stream. Their permeate stream has volume R at concentration $\overline{C}_p$ whereas the feed stream has unit volume at concentration C_f. The concentrate will have volume (1-R) and the concentrate concentration, C_c, according to mass balance information will be

$$\frac{C_c}{C_f} = \frac{1 - R(\overline{C}_p/C_f)}{1 - R} \qquad \text{(B9)}$$

When each stream is subjected to bioassay a set of dilutions is determined which produce effects on half the population. These dilutions are δ_p, δ_f, and δ_c for the permeate, feed, and concentrate, respectively. The dilution δ is the fraction of sample in a unit of total fluid, so that $\delta \equiv LC_{50}$. One expects the single solute to produce a medium effect at a concentration, C_{50}, independent of permeate or feed or concentrate source

$$C_{50} = \delta_p \overline{C}_p = \delta_c C_c = \delta_f C_f$$

Solving for each individual concentration value

$$\overline{C}_p = \frac{C_{50}}{\delta_p}$$

$$C_c = \frac{C_{50}}{\delta_c} \qquad \text{(B10)}$$

$$C_f = \frac{C_{50}}{\delta_f}$$

The concentration of toxicant is seen to be inversely proportional to the value of δ ($\delta = LC_{50}$). Substitution of each value from equations (B10) into relation (B9) yields

$$\frac{\delta_f}{\delta_c} = \frac{1 - R(\delta_f/\delta_p)}{1 - R} \qquad \text{(B11)}$$

Equation (B11) is a kind of mass balance for the toxicant. Written as a ratio of solute mass in permeate and concentrate to solute mass in feed the mass balance ratio is

$$\text{M.B.R.} = \delta_f(R/\delta_p + [1-R]/\delta_p) \qquad \text{(B12)}$$

Equation (B11) is useful in predicting the value of δ_c knowing δ_p, δ_f, and R while the ratio in equation (B12) is useful in evaluating the internal consistency of the data for all parameters. As noted in the report the mass balance ratios were ± 60 percent of unity, which is felt to be very reasonable for biological assay data.

Example for calculation of mass balance ratio for Run 1 with Fathead minnows.

L	LC_{50} (Table 11)	Concentration (Table 11)	Volumes
feed	9.7	10.	1
PEA permeate	82	1.2	0.379
CA permeant	>100	<1	0.484
concentrate	1.6	62.	0.137

mass of toxicant in concentrate 0.137 x 62 = 8.494
in PEA permeate 0.379 x 1.2 = 0.4548
in CA permeate 0.484 x (<1) = 0.48
Total end of run = 9.43

in feed 1 x 10 = 10

$$\text{mass balance ratio} = \frac{9.4}{10} = 0.94$$

this value is shown as the first entry in Table 13.

APPENDIX C

EVALUATION OF HYPERFILTRATION TREATED TEXTILE WASTEWATERS

by

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SECTION 1

INTRODUCTION

On 7 June 1976, the U.S. District Court of Washington, D.C., issued a consent decree (resulting from Natural Resources Defense Council, et al. versus Train) requiring EPA to accelerate development of effluent standards for 21 industrial point sources including textile manufacturing. Among other requirements, the Court's mandate focused federal water pollution control efforts on potentially toxic and hazardous chemical compounds. The consent decree required that "65 classes" of chemical compounds be analyzed in wastewater samples. Recognizing the difficulty of analyzing for all chemical species present in each category of compounds, EPA developed a surrogate list of 129 specific compounds representative of the classes of compounds listed in the consent decree. These compounds are referred to as "priority pollutants."

The consent decree obligates EPA to identify which priority pollutants are present in industrial wastewaters and to determine the ability of various wastewater treatment technologies to remove priority pollutants. It is the second item above to which this project is directed. Under EPA Grant No. R805777, Clemson University is evaluating the ability of a hyperfiltration unit to treat textile manufacturing wastewaters. Samples of two wastewater feeds and hyperfiltration permeates and concentrates using three types of membranes were sent to Monsanto Research Corporation (MRC) for priority pollutant analysis and bioassay testing. The following bioassay tests were performed to evaluate the reduction in toxicity by hyperfiltration of wastewater: Ames mutagenicity and cytotoxicity tests, and fathead minnow, daphnia, and 14-day rat acute toxicity tests.

This report discusses the analytical and bioassay procedures used by MRC and its subcontractors and the results of the analyses.

SECTION 2

SUMMARY

Under EPA Grant No. R805777, researchers J. L. Gaddis and H. G. Spencer at Clemson University are evaluating the effectiveness of hyperfiltration to cleanup various textile plant wastewaters for discharge and possible recycle of chemical feedstocks.

The skid-mounted hyperfiltration unit was field tested on two types of wastewater (scour bath and dye waste) at a textile woven fabric finishing plant. Three types of hyperfiltration membranes were tested: polyether amide (PEA), cellulose acetate (CA), and dual-layer hydrous zirconium oxide (ZrO)-polyacrylate (PAA) dynamic membrane.

A total of 16 wastewater samples consisting of the hyperfiltration feed, permeate, and concentrate were sent to MRC for priority pollutant analysis and bioassay testing. The sample coding system and corresponding description of the sample collected is shown in Table 1. MRC performed the priority pollutant analyses, Ames mutagenicity test, and cytotoxicity test (using Chinese hamster ovary - CHO cells). Fathead minnow and daphnia acute toxicity tests were performed for MRC by EG&G Bionomics Marine Research Laboratory. The 14-day rat acute toxicity tests were performed for MRC by Litton Bionetics.

Results of the analysis of 16 wastewater samples and a reagent blank for the presence of the 114 organic priority pollutants are shown in Table 2. Analysis of the data indicates no organic priority pollutants are introduced due to the sample workup procedures or analysis contamination at MRC. Samples CTHF-1 and CTHF-2 were samples of the textile plant intake water and hyperfiltration unit rinse. Analyses of these samples indicate that possibly chloroform and toluene are introduced from these two sources.

In addition to the organic priority pollutant species, several other organic compounds were detected in the wastewater samples. Triphenyl phosphine and triphenyl phosphine oxide were detected in all wastewater samples (except CTHF-6) and in the reagent blank sample. These compounds probably result from glass cleaning detergents and were introduced from the sample containers and laboratory glassware. Other organic compounds detected include:

TABLE C1. SAMPLE CODING SCHEME AND DESCRIPTION OF SAMPLE COLLECTED

Sample	Description
CTHF-1	Plant water
CTHF-2	Apparatus water
CTHF-3	Scour-1, feed for PEA and CA hyperfiltration
CTHF-4	Scour-1, permeate from PEA hyperfiltration
CTHF-5	Scour-1, permeate from CA hyperfiltration
CTHF-6	Scour-1, concentrate from PEA and CA hyperfiltration
CTHF-7	Scour-2, feed for DM hyperfiltration
CTHF-8	Scour-2, permeate from DM hyperfiltration
CTHF-9	Scour-2, concentrate from DM hyperfiltration
CTHF-10	Dye-1, feed for PEA and CA hyperfiltration
CTHF-11	Dye-1, permeate from PEA hyperfiltration
CTHF-12	Dye-1, permeate from CA hyperfiltration
CTHF-13	Dye-1, concentrate from PEA and CA hyperfiltration
CTHF-14	Dye-2, feed for DM hyperfiltration
CTHF-15	Dye-2, permeate from DM hyperfiltration
CTHF-16	Dye-2, concentrate from DM hyperfiltration

α-terepineol, 2-mercaptobenzthiazole, 1-cyano-2-benzyloxy ethane, benzthiazole, lauric acid, myristic acid, palmitic acid, and stearic acid. Results of the priority pollutant metals analysis for the 16 wastewater samples are shown in Table 3. Three priority pollutant metals (mercury, selenium, and thallium) were not analyzed in this program because previous research indicated the absence of these metals in textile plant wastewaters.

Because of the metals analytical technique used, 16 other trace metals were analyzed in the samples: aluminum, barium, boron, calcium, cobalt, iron, magnesium, manganese, molybdenum, phosphorus, silicon, sodium, strontium, tin, titanium, and vanadium.

Results of the phenol (total) and cyanide (total) analyses are also shown in Table 3.

Fourteen of the sixteen wastewater samples (excluding CTHF-1 and CTHF-2) were subjected to a battery of bioassay tests to determine the reduction in toxicity by application of hyperfiltration to various wastewaters. MRC performed the Ames mutagenicity test and CHO cytotoxicity test on the samples. MRC directed Clemson University to ship samples to EG&G Bionomics Marine Research Laboratory, Wareham, Massachusetts, for freshwater static acute toxicity tests using fathead minnows (*Pimephales promelas*) and daphnids (*Daphnia magna*). Samples were likewise sent to Litton Bionetics, Kensington, Maryland, for 14-day rat acute toxicity testing.

TABLE C2. ORGANIC PRIORITY POLLUTANT SPECIES DETECTED IN SPECIFIC WASTEWATER STREAMS
($\mu g/\ell$)

Organic compound	Blank water	Concentration in stream CTHF-1	CTHF-2	CTHF-3	CTHF-4	CTHF-5	CTHF-6	CTHF-7	CTHF-8
Bis(2-ethylhexyl)phthalate	1.1		9	9	3	3		9	
Dimethyl phthalate			18		9				
Di-n-butyl phthalate	0.4			4	1			3	
Butylbenzyl phthalate									
Diethyl phthalate	0.3								
Acenaphthene				7	0.8			7	
Anthracene				2					
Fluoranthene				0.4					
Pyrene				1					
Naphthalane					0.5				
Phenanthrene								2	
Phenol			0.9		2	3	13		
Chloroform		58	31	18	18	22		34	
Toluene		3	22	0.8	15	29	41	0.8	0.7
Trichloroethylene				0.3		0.4	5		2
Benzene			2		1	1	6		2
Chlorobenzene						0.7			
Ethylbenzene							21		
Methylene chloride		6	34	5	6	5	15	4	5

(continued)

Note.—Blanks indicate compound is below detection limits.

TABLE C2 (continued)

Organic compound	Concentration in stream CTHF-9	CTHF-10	CTHF-11	CTHF-12	CTHF-13	CTHF-14	CTHF-15	CTHF-16
Bis(2-ethylhexyl)phthalate		4	31	3	51	2	1	4
Dimethyl phthalate		55	45		290	170	4	
Di-n-butyl phthalate		1	0.8		6	1	1	1
Butylbenzyl phthalate			1		7			
Diethyl phthalate							0.05	
Acenaphthene		3	0.8		7	3		
Anthracene		0.6			3	0.7	0.1	
Fluoranthene						0.1		
Pyrene								
Naphthalene		0.8				0.8		
Phenanthrene								
Phenol	1	0.2	0.7	0.4	1	0.2		
Chloroform		19	31	4		96		
Toluene	0.5	10	11	24		0.6	0.4	1
Trichloroethylene			0.6			0.6	1	
Benzene	0.7	2	0.4	1				
Chlorobenzene								
Ethylbenzene								
Methylene chloride	2	5	45	4	14	5	3	3

Note.—Blanks indicate compound is below detection limits.

TABLE C3. CONCENTRATION OF PRIORITY POLLUTANT METALS, PHENOL, AND CYANIDE DETECTED IN SPECIFIC WASTEWATER STREAMS ($\mu g/\ell$)

Species	Detection limit	Concentration in stream							
		CTHF-1	CTHF-2	CTHF-3	CTHF-4	CTHF-5	CTHF-6	CTHF-7	CTHF-8
Priority pollutant metal species:									
Antimony	10	12	30	100	90	132	436	170	146
Arsenic	2	<1	<1	19	<1	1	160	35	5
Beryllium	0.04	-	-	-	-	-	-	-	-
Cadmium	2	5	9	15	15	14	48	16	20
Chromium	4	540	840	640	720	620	1,260	760	800
Copper	4	54	200	90	26	32	760	94	
Lead	22	168	240	380	250	340	760	400	414
Nickel	36	-	154	132	70	100	480	200	210
Silver	5	-	24	42	26	42	114	62	78
Zinc	1	630	616	520	360	8,600	3,540	460	248
Other species:									
Phenol (total)	1	1	33	6	12	16	-[a]	4	<1
Cyanide (total)	1	<7	4	<4	72	30	-[a]	<4	<7

(continued)

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[a] Sample arrived at MRC 4 days after sample collection and at room temperature; therefore, no analysis was performed due to poor sample integrity.

TABLE C3 (continued)

Species	Concentration in stream							
	CTHF-9	CTHF-10	CTHF-11	CTHF-12	CTHF-13	CTHF-14	CTHF-15	CTHF-16
Priority pollutant metal species:								
Antimony	380	192	132	116	280	196	160	320
Arsenic	∿14	35	15	<1	221	2	<1	9
Beryllium	-	-	-	34	-	-	-	-
Cadmium	120	22	20	48	40	20	34	70
Chromium	1,040	540	760	520	1,000	900	680	1,320
Copper	644	480	46	50	3,060	10,600	100	35,400
Lead	920	520	404	380	700	520	450	1,140
Nickel	468	220	200	62	480	186	220	600
Silver	200	82	68	20	116	70	84	126
Zinc	6,560	7,200	360	140	5,360	6,600	188	12,800
Other species:								
Phenol (total)	13	19	20	18	64	12	3	26
Cyanide (total)	62	<1	<1	<1	8	<4	<4	20

Results of the bioassays are shown in Table 4. None of the samples were mutagenic in the Ames test in the range of sample concentrations tested - 10 to 1,000 μℓ/plate. Two samples (CTHF-6 and 13) indicated acute toxicity to CHO cells. Sample CTHF-16 exhibited acute toxicity but in a sample concentration higher than that tested. Analysis of the fathead minnow and daphnia acute toxicity data indicated the four permeate samples (CTHF-5, 8, 12 and 15) produced no or very little mortality. The most toxic samples were the concentrates (CTHF-6, 9, 13, and 16).

Data from the 14-day rat test indicated that no rat deaths or sample related physical effects occurred due to a single maximum dosage. Therefore, no samples were subjected to the quantitative bioassay.

TABLE C4. SUMMARY OF BIOASSAY TEST RESULTS

Sample	Microbial mutagenicity response	Cytotoxicity, EC_{50} (% waste-water solution)	Daphnia acute toxicity, LC_{50} (% wastewater solution)	Fathead minnow acute toxicity, LC_{50} (% wastewater solution)	Rat acute toxicity, LD_{50} (g-sample/kg body weight)
CTHF-3	Negative	NAT[a]	26 (20 to 34)[b]	16 (13 to 21)	>10
CTHF-4	Negative	NAT	53 (45 to 62)	28 (24 to 33)	>10
CTHF-5	Negative	NAT	42 (35 to 51)	-[c]	>10
CTFH-6	-[d]	9	5.1 (4.2 to 6.2)	1.5 (1.0 to 2.2)	>10
CTHF-7	Negative	NAT	25 (20 to 31)	13 (7.8 to 22)	>10
CTHF-8	Negative	NAT	>100	NAT	>10
CTHF-9	Negative	NAT	9.9 (8.3 to 12)	2.0 (1.5 to 2.8)	>10
CTHF-10	Negative	NAT	33.5 (27.6 to 50.4)	9.7 (7.5 to 12)	>10
CTHF-11	Negative	NAT	>60 <100[e]	82 (21 to 100)	>10
CTHF-12	Negative	NAT	>60 <100	NAT	>10
CTHF-13	Negative	10	4.1 (3.4 to 4.9)	1.6 (1.2 to 2.0)	>10
CTHF-14	Negative	NAT	49 (41 to 58)	25 (21 to 39)	>10
CTHF-15	Negative	NAT	80 (71 to 90)	NAT	>10
CTHF-16	Negative	>20	17 (12 to 23)	5.3 (4.1 to 6.8)	>10

[a] No acute toxicity.

[b] Values in parentheses are 95% confidence intervals.

[c] Only 30% mortality occurred in 100% solution of wastewater.

[d] CTHF-6 could not be readily filter sterilized, therefore the Ames test could not be performed.

[e] >60 <100 means LC_{50} value is greater than 60% but less than 100%.

SECTION 3

SAMPLE COLLECTION

Hyperfiltration is a separation process involving the filtering of aqueous solutions by membranes capable of removing not only suspended particles but also substantial fractions of dissolved impurities, including organic and inorganic material. The process is illustrated schematically in Figure 1. Application of high pressure to the feed solution causes purified permeate water to pass through the membrane. Remaining feed water becomes a concentrated solution of suspended solids and higher molecular weight compounds.

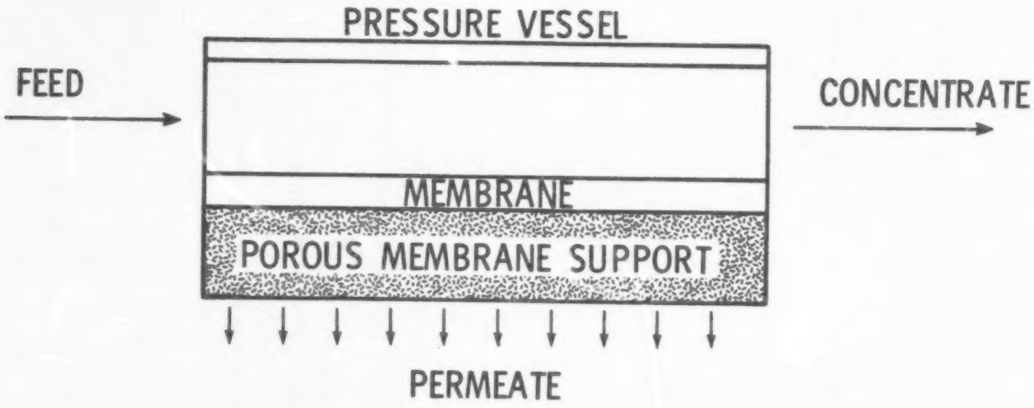

Figure C1. Schematic diagram of a hyperfiltration module.

In the Clemson University study, EPA Grant No. 805777, two wastewater streams were used as feed: 1) scour bath wastewater, and 2) wastewater from dying operations. In addition, three hyperfiltration membranes were tested on each wastewater: 1) polyether amide (PEA) membrane, 2) cellulose acetate (CA) hyperfilter, and 3) a dynamic membrane (DM) of a dual-layer hydrous Zr(IV) oxide-polyacrylate. The polyether amide and cellulose acetate membranes were tested in series, resulting in two permeate samples and one concentrate sample per feed tested. The resulting sample coding system and volume of sample collected in the test program are shown in Table 5.

TABLE C5. COLLECTION SAMPLES FOR BIOASSAY TESTS AND CHEMICAL ANALYSES

Sample	Description	Volume, gal
CTHF-1	Plant water	5
CTHF-2	Apparatus water	5
CTHF-3	Scour-1, feed for PEA and CA hyperfiltration	25
CTHF-4	Scour-1, permeate from PEA hyperfiltration	25
CTHF-5	Scour-1, permeate from CA hyperfiltration	25
CTHF-6	Scour-1, concentrate from PEA and CA hyperfiltration	10[a]
CTHF-7	Scour-2, feed for DM hyperfiltration	25
CTHF-8	Scour-2, permeate from DM hyperfiltration	25
CTHF-9	Scour-2, concentrate from DM hyperfiltration	10[a]
CTHF-10	Dye-1, feed for PEA and CA hyperfiltration	25
CTHF-11	Dye-1, permeate from PEA hyperfiltration	25
CTHF-12	Dye-1, permeate from CA hyperfiltration	25
CTHF-13	Dye-1, concentrate from PEA and CA hyperfiltration	10[a]
CTHF-14	Dye-2, feed for DM hyperfiltration	25
CTHF-15	Dye-2, permeate from DM hyperfiltration	25
CTHF-16	Dye-2, concentrate from DM hyperfiltration	10[a]

[a] Concentrate samples will be 2 gal to 5 gal, containing equivalent solids to the feed sample.

Sample CTHF-1 was the textile plant intake water. The hyperfiltration unit was cleaned of residual materials using a sequence of washes. A detergent wash followed by a caustic wash removed residual greases, waxes, and organic materials. A nitric acid wash followed to remove trace metals from the stainless steel surfaces. The unit was then rinsed with plant intake water. The unit was finally operated for several hours with plant water to indicate whether materials were evolved within the plumbing. Sample CTHF-2 was a sample of this water.

Samples generated in the testing program were analyzed for the 129 priority pollutants and subjected to five bioassay tests. The priority pollutant analysis scheme is divided into the following fractions for sampling purposes: volatile organics, nonvolatile organics, metals, cyanide (total), and phenol (total). Three separate samples were required for bioassay testing: 1) microbical mutagenicity (Ames test) and cytotoxicity, 2) 14-day rat acute toxicity test, and 3) freshwater static acute toxicity test with fathead minnows and daphnids. Samples for priority pollutant analysis, Ames test, and cytotoxicity tests were sent by Clemson University directly to MRC for analysis. To expedite sampling delivery and insure sample integrity, MRC directed Clemson University to ship the remaining samples for bioassay testing directly to the testing laboratories.

Table 6 shows the sample fractions collected, volume, and containers used for each stream sampled. Note that the plant intake water (CTHF-1) and hyperfiltration rinse water (CTHF-2) were not subjected to bioassay testing.

TABLE C6. BIOASSAY TESTS AND CHEMICAL ANALYSES, TEST-SAMPLE CONTAINERS, AND TESTS DESIGNATED FOR COLLECTION SAMPLES

Test No.	Description	Sample volume	Container	Required for collection samples (CTHF)
B.1	Microbial mutagenicity (Ames) and cytotoxicity (hamster ovary cells)	500 mℓ	Amber glass, Teflon-lined caps	3 to 16
B.2	Acute toxicity (rat)	500 mℓ	Glass, Teflon-lined caps	3 to 16
B.3	Freshwater static bioassay (Daphnia and fathead minnows)	20 gal[a]	5 gallon, plastic cubitainers	3 to 16
C.1	Volatile solutes	2 x 40 mℓ	Amber glass vials, Teflon-lined septa	1 to 16
C.2	Nonvolatile solutes	2 x 1 gal	Amber glass, Teflon-lined caps	1 to 16
C.3	Metals	500 mℓ	Plastic bottles	1 to 16
C.4	Cyanide	500 mℓ	Plastic bottles	1 to 16
C.5	Phenols	500 mℓ	Amber glass	1 to 16
C.6	Pesticides	(use part of test sample C.2)		

[a] Concentrate samples will be 2 gal to 5 gal, containing equivalent solids to the feed sample.

SECTION 4

PRIORITY POLLUTANT ANALYSIS

ANALYTICAL PROCEDURE

Analyses of the 16 wastewater samples for the 129 priority pollutants were performed by MRC in accordance with the analytical methodology recommended by EPA (1). It is important to realize that the purpose of EPA's analytical scheme is to screen samples to determine which of the 129 chemical species are present and to estimate their general concentration range. Currently, the recommended analytical protocol is in the developmental stage and requires further verification and validation. Analytical results must be considered as reliable estimates of which priority pollutants were present, with concentrations accurate to within a factor of two.

Of the 129 priority pollutants, two species were not determined in this project: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and asbestos. EPA-Environmental Monitoring and Support Laboratory (EMSL) recommended that TCDD should be omitted because of its extreme toxicity and potential health hazard involved in preparing standard solutions from the pure compound (1). Asbestos was eliminated, as recommended by the EPA Project Officer.

Priority pollutants are divided into the following fractions for analysis purposes: volatile organics, base/neutral organics, acid organics, pesticides, polychlorinated biphenyls (PCB), metals, phenol (total), and cyanide (total) (1).

A brief discussion of the analysis procedures used and sample analysis results are given in the following three subsections.

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(1) Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. Draft final report, U.S. Environmental Protection Agency, Cincinnati, Ohio, April 1977. 145 pp.

Volatile Organics

The recommended method for volatile organic analysis was designed by EPA to determine those chemical species which were amenable to the Bellar purge and trap method (1). Appendix A lists those priority pollutants classified as volatile organics.

Two hermatically sealed 40-mℓ glass vials collected from each of the 16 samples were composited in the laboratory for one analysis. Two composited solutions were used, one for analysis and one as a backup sample. Figure 2 is a simplified diagram of the analytical scheme for volatile organics analysis.

An internal standard of 1,4-dichlorobutane was added to 5 mℓ of the composited sample and the sample sparged with helium onto a Tenax GC-silica-packed sample tube. Two tubes were prepared, one for analysis and one duplicate. Tenax tubes were then sealed in glass under a nitrogen atmosphere and stored in a freezer at -18°C until analyzed.

Analyses were carried out using a Hewlett Packard 5981 GC-Mass Spectrometer with 5934 Data System. Sample tubes were heated to 180°C over a 1-min period and held at that temperature for 4 min to desorb the compounds onto a Carbowax 1500 column held at -40°C. For compounds with boiling points below room temperature, cryogenic trapping at -40°C (liquid nitrogen cooling) was found to give better reproducibility of retention time than using the suggested temperature of 30°C. After desorption, the GC column temperature was raised 8°C/min to 170°C.

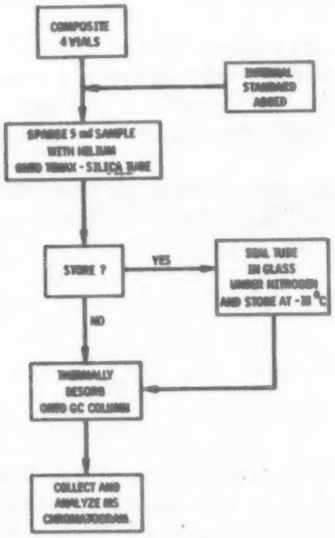
COMPOSITE 4 VIALS
INTERNAL STANDARD ADDED
SPARGE 5 mℓ SAMPLE WITH HELIUM ONTO TENAX - SILICA TUBE
STORE ?
YES
SEAL TUBE IN GLASS UNDER NITROGEN AND STORE AT -18 °C
NO
THERMALLY DESORB ONTO GC COLUMN
COLLECT AND ANALYZE MS CHROMATOGRAM

Figure 2. Analytical scheme for volatile organics analysis.

Qualitative identification of a compound was made using three criteria listed in the protocol (1): 1) retention time must coincide with known retention times, 2) three characteristic masses must elute simultaneously, and 3) intensities of the characteristic masses must stand in the known proper proportions. Quantitation of volatile organics were made using response ratios of the 1,4-dichlorobutane internal standard.

Nonvolatile Organics

Nonvolatile organics are divided into three groups for analysis: base/neutral fraction, acid fraction, and pesticides and polychlorinated biphenyls (PCB). A list of compounds that are classified as nonvolatile organics is given in Appendix A.

The analytical procedure is described in Reference 1. Figure 3 depicts the sample processing scheme for the base/neutral and acid fractions. The sample solution, 2 ℓ, was made alkaline (pH greater than 11) with sodium hydroxide, and then extracted three times with methylene chloride. The wastewater samples formed emulsions upon extraction with methylene chloride. The problem was resolved by drawing off small amounts of separated solvent and pouring the extract through the sample in the separatory funnel. Separation was also enhanced by slowly dripping the emulsion onto the wall of a slightly tilted flask.

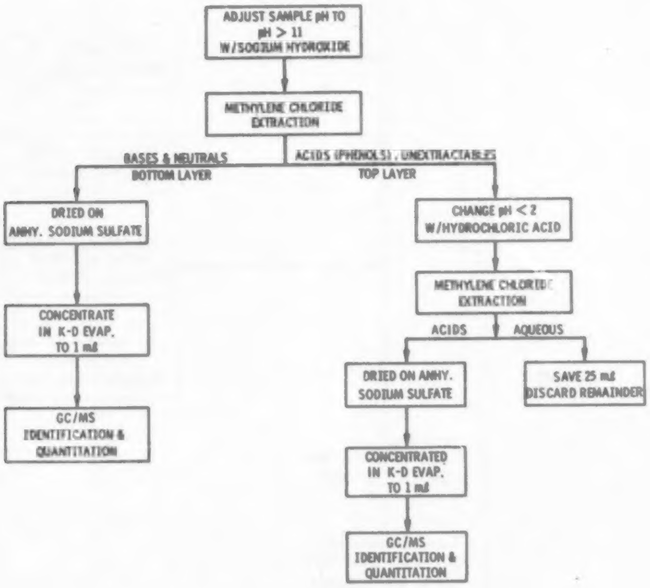
ADJUST SAMPLE pH TO pH > 11 W/SODIUM HYDROXIDE
METHYLENE CHLORIDE EXTRACTION
BASES & NEUTRALS BOTTOM LAYER
ACIDS (PHENOLS), UNEXTRACTABLES TOP LAYER
DRIED ON ANHY. SODIUM SULFATE
CONCENTRATE IN K-D EVAP. TO 1 mℓ
GC/MS IDENTIFICATION & QUANTITATION
CHANGE pH < 2 W/HYDROCHLORIC ACID
METHYLENE CHLORIDE EXTRACTION
ACIDS
AQUEOUS
DRIED ON ANHY. SODIUM SULFATE
SAVE 25 mℓ DISCARD REMAINDER
CONCENTRATED IN K-D EVAP. TO 1 mℓ
GC/MS IDENTIFICATION & QUANTITATION

Figure 3. Sample processing scheme for nonvolatile organics analysis.

Extracts were dried on a column of anhydrous sodium sulfate, concentrated to 1.0 mℓ in a Kuderna-Danish (K-D) evaporator with a Snyder column, spiked with deuterated anthracene, sealed in septum capped vials, and stored at 4°C until analyzed. Analyses were performed on the GC-MS system using SP 2250 and Tenax GC columns for base/neutral and acid samples, respectively (1).

A separate 1.0 ℓ sample was used for analysis of the pesticides and PCB (Aroclor® fluids). The basic sample processing scheme is shown in Figure 4. These compounds were extracted with a 15% methylene chloride and 85% hexane solvent mixture. The aqueous phase was discarded, and the organic phase was analyzed by GC with an electron capture detector. Where necessary, acetonitrile partitioning and a Florisil® chromatography column were used for further cleanup of the sample. All samples went through acetonitrile partitioning cleanup, only.

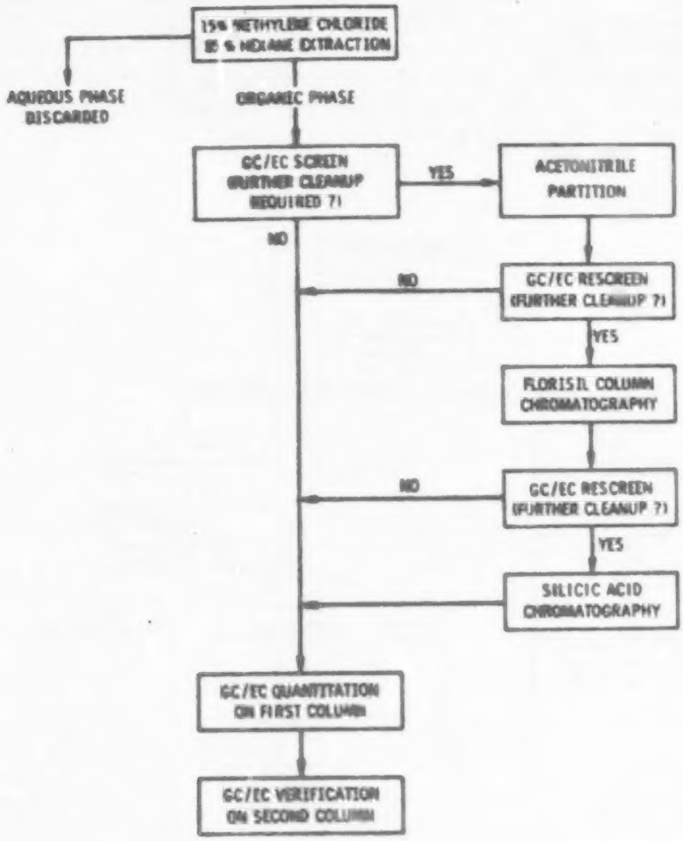
15% METHYLENE CHLORIDE
85 % HEXANE EXTRACTION
AQUEOUS PHASE
DISCARDED
ORGANIC PHASE
GC/EC SCREEN
(FURTHER CLEANUP
REQUIRED ?)
YES
ACETONITRILE
PARTITION
NO
NO
GC/EC RESCREEN
(FURTHER CLEANUP ?)
YES
FLORISIL COLUMN
CHROMATOGRAPHY
NO
GC/EC RESCREEN
(FURTHER CLEANUP ?)
YES
SILICIC ACID
CHROMATOGRAPHY
GC/EC QUANTITATION
ON FIRST COLUMN
GC/EC VERIFICATION
ON SECOND COLUMN

Figure 4. Sample processing scheme for pesticide and PCB analysis.

Confirmation of identity and quantitation were made using two different GC columns: SP-2550 and Dexil 410. Compound verification was made with the MS when the concentration was greater than 10 μg/ℓ. Concentrations of potential pesticides ranged from 0.1 μg/ℓ to 10 μg/ℓ; therefore, MS verification was not possible in this study. Pesticide species identified only by GC below 10 μg/ℓ were reported only if they met the following two criteria: 1) the retention time window between standards and unknown peaks correlated within ±3 s, and 2) concentrations calculated from both GC columns had to agree within ±20%. Unknown peaks not meeting these criteria were assumed not to be the pesticide species.

Metals

In addition to the volatile and nonvolatile organics, the 129 chemical species include 13 metals, measured as the total metal. Sixteen metal samples were collected and shipped in low-density polyethylene plastic bottles. Due to U.S. Department of Transportation regulations regarding air freight of hazardous materials, the samples were not acidified in the field. Upon arrival at MRC, 5 mℓ of redistilled nitric acid (HNO_3) were added to each sample and the sample allowed to stand for 24 hr before processing.

Each metals sample was beaker digested to reduce sample matrix effects with HNO_3 for about 6 hr or until the solution became clear. The digested solution was then taken up to 100 ml with distilled deionized water and stored in low-density polyethylene plastic bottles.

The following nine priority pollutant metals were analyzed on the Jarrell-Ash Plasma Atomcomp, Model 975 with inductively coupled argon plasma excitation (ICAP) at Monsanto Company's Physical Sciences Center in St. Louis: antimony, beryllium, cadmium, chromium, copper, lead, nickel, silver, and zinc. ICAP is an optical emission spectroscopy analytical system for simultaneous multi-element determination of trace metals. In this device, a stream of inert gas (argon) is first ionized and then a concentric, which is a source of a high frequency (HF) field, accelerates the electrons until they acquire sufficient energy to excite and ionize atoms. The elements of the wastewater samples introduced into this plasma are immediately raised to a higher energy state from which they decay with ultraviolet (uv), visible, and infrared (ir) emissions.

Of the remaining four priority pollutant metals, only arsenic was measured in the 16 samples because previous research indicated that mercury, selenium, and thallium were not in textile

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(2) Rawlings, G. D. Source Assessment: Textile Plant Wastewater Toxics Study - Phase I. EPA-600/2-78-004h, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, March 1978. 166 pp.

wastewaters (2). Arsenic was measured by conventional atomic absorption techniques in accordance with Referances 3 and 4.

In addition to the 16 samples, 5 other samples were included as part of the quality assurance program. A certified U.S. National Bureau of Standards trace metal in water standard (No. 1643) was included with the set of samples. Two trace metal standards prepared by MRC were included: one concentrated standard and one 5 mℓ/ℓ dilute standard. A separate standard was added for silver and nickel quality assurance testing. Finally, one of the real samples was split and submitted as a blind repeat.

Since ICAP simultaneously analyzes for 25 trace elements, the results of the nonpriority pollutant metals is also reported.

Cyanide (Total)

Total cyanide was analyzed according to the procedure in Reference 1. Two standard solutions were prepared and sent with the samples along with two blind repeats of the standards.

Phenol (Total)

In addition to specific phenolic compounds and phenol measured by GC-MS in the acid fraction, total phenol was also measured by typical wet chemistry techniques (1, 3, 4).

Phenol samples were preserved in the field by adding 1.0 g $CuSO_4$; maintaining the pH to less than 4 with H_3PO_4 and storing the sample at 4°C. Recent research has indicated this preservation technique is adequate for at least 8 days (5). All phenolic samples collected in this study were analyzed within 5 days of collection.

RESULTS OF CHEMICAL ANALYSIS

Organic Species

Results of the analysis of 16 wastewater samples for the presence of the 114 organic priority pollutant species are shown in

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(3) Manual of Methods for Chemical Analysis of Water and Wastes. EPA-625/6-76-003a (PB 259 973) U.S. Envionmental Protection Agency, Cincinnati, Ohio, 1976. 317 pp.

(4) Standard Methods for the Examination of Water and Wastewater, Fourteenth Edition. American Public Health Association, Washington, D.C., 1976. 874 pp.

(5) Carter, M. J., and M. T. Huston. Preservation of Phenolic Compounds in Wastewaters. Environmental Science and Technology, 12(3):309-313, 1978.

Table 7. A reagent blank using organic free water was included with the samples and worked up and analyzed like all the samples. Results of this analyses are shown in the second column of Table 7.

Analysis of the data indicates no organic priority pollutants are introduced due to the sample workup reagents or analysis contamination at MRC. Samples CTHF-1 and CTHF-2 were samples of the textile plant intake water and hyperfiltration unit rinse. Analyses of these samples indicate that possibly chloroform and toluene are introduced from these two sources. The remaining organic priority pollutant species in Table 7 are present in the wastewater samples.

Samples with the largest number of organic species are the four feed streams (CTHF-3, 7, 10, and 14). Species found in the concentrate and not found in the feed are due to the concentrating mechanism of the hyperfiltration unit and the species are now above detection limits. The detection limit for the 114 organic priority pollutants are shown in Table 8.

In addition to the organic priority pollutant species, several other organic compounds were detected in the samples, Table 9. These compounds were identified by their characteristic fragmentation pattern in the mass spectrometer based on their principle ion and corresponding mirror ions. Qualitative concentration values were determined based on the peak heights of known concentrations of priority pollutants which elute the gas chromatograph in adjacent retention time windows.

Analysis of the data indicate that triphenyl phosphine and its oxide are probably a result of glass cleaning detergents and was introduced from the sample containers and laboratory glassware.

Metals

Results of ICAP and atomic absorption analyses of the 16 digested metals samples are shown in Tables 10 and 11. Table 10 shows the priority pollutant metals, while Table 11 shows the other metals simultaneously measured by ICAP. Note that the second column in both tables shows the detection limit for each metal.

Results of the trace metals quality assurance program are presented in Table 12.

Phenol (Total) and Cyanide (Total)

Results of the phenol (total) and cyanide (total) analyses were also presented in Table 10. Sample CTHF-6 arrived at MRC four days after sample collection and at room temperature. Therefore, total phenol and cyanide were not measured due to poor sample integrity.

TABLE C7. ORGANIC PRIORITY POLLUTANT SPECIES DETECTED IN SPECIFIC WASTEWATER STREAMS (µg/ℓ)

Organic compound	Blank water	Concentration in stream							
		CTHF-1	CTHF-2	CTHF-3	CTHF-4	CTHF-5	CTHF-6	CTHF-7	CTHF-8
Bis(2-ethylhexyl)phthalate	1.1		9	9	3	3		9	
Dimethyl phthalate			18		9				
Di-n-butyl phthalate	0.4			4	1			3	
Butylbenzyl phthalate									
Diethyl phthalate	0.3								
Acenaphthene				7	0.8			7	
Anthracene				2					
Fluoranthene				0.4					
Pyrene				1					
Naphthalane					0.5				
Phenanthrene								2	
Phenol			0.9		2	3	13		
Chloroform		58	31	18	18	22		34	
Toluene		3	22	0.8	15	29	41	0.8	0.7
Trichloroethylene				0.3		0.4	5		2
Benzene			2		1	1	6		2
Chlorobenzene						0.7			
Ethylbenzene							21		
Methylene chloride		6	34	5	6	5	15	4	5

(continued)

Note.—Blanks indicate compound is below detection limits.

TABLE C7. (continued)

Organic compound	Concentration in stream							
	CTHF-9	CTHF-10	CTHF-11	CTHF-12	CTHF-13	CTHF-14	CTHF-15	CTHF-16
Bis(2-ethylhexyl)phthalate		4	31	3	51	2	1	4
Dimethyl phthalate		55	45		290	170	4	
Di-n-butyl phthalate		1	0.8		6	1	1	1
Butylbenzyl phthalate			1		7			
Diethyl phthalate							0.05	
Acenaphthene		3	0.8		7	3		
Anthracene		0.6			3	0.7	0.1	
Fluoranthene						0.1		
Pyrene								
Naphthalene		0.8				0.8		
Phenanthrene								
Phenol	1	0.2	0.7	0.4	1	0.2		
Chloroform		19	31	4		96		
Toluene	0.5	10	11	24		0.6	0.4	1
Trichloroethylene			0.6			0.6	1	
Benzene	0.7	2	0.4	1				
Chlorobenzene								
Ethylbenzene								
Methylene chloride	2	5	45	4	14	5	3	3

Note.—Blanks indicate compound is below detection limits.

TABLE C8. MINIMUM DETERMINABLE CONCENTRATIONS (μg/ℓ)

Compound	Detection limit	Compound	Detection limit
Acids:		**Base neutrals:**	
2-Chlorophenol	0.09	1,3-Dichlorobenzene	0.02
Phenol	0.07	1,4-Dichlorobenzene	0.04
2,4-Dichlorophenol	0.1	Hexachloroethane	0.1
2-Nitrophenol	0.4	1,2-dichlorobenzene	0.05
p-Chloro-*m*-cresol	0.1	Bis(2-chloroisopropyl)ether	0.06
2,4,6-Trichlorophenol	0.2	Hexachlorobutadiene	0.08
2,4-Dimethylphenol	0.1	1,2,4-Trichlorobenzene	0.09
2,4-Dinitrophenol	2.0	Naphthalene	0.007
4,6-Dinitro-*o*-cresol	40.0	Bis(2-chloroethyl)ether	0.07
4-Nitrophenol	0.9	Hexachlorocyclopentadiene	0.2
Pentachlorophenol	0.4	Nitrobenzene	0.08
		Bis(2-chloroethoxy)methane	0.06
Volatiles:		2-Chloronaphthalene	0.02
Chloromethane	0.2	Acenaphthylene	0.02
Dichlorodifluoromethane	0.2	Acenaphthene	0.04
Bromomethane	0.2	Isophorone	0.06
Vinyl chloride	0.4	Fluorene	0.02
Chloroethane	0.5	2,6-Dinitrotoluene	0.2
Methylene chloride	0.4	1,2-Diphenylhydrazine	0.02
Trichlorofluoromethane	2.0	2,4-Dinitrotoluene	0.02
1,1-Dichloroethylene	2.0	N-nitrosodiphenylamine	0.07
1,1-Dichloroethane	3.0	Hexachlorobenzene	0.05
Trans-1,2-Dichloroethylene	2.0	4-Bromophenyl phenyl ether	0.1
Chloroform	5.0	Phenanthrene	0.01
1,2-Dichloroethane	2.0	Anthracene	0.01
1,1,1-Trichloroethane	2.0	Dimethyl phthalate	0.03
Carbon tetrachloride	4.0	Diethylphthalate	0.03
Bromodichloromethane	0.9	Fluoranthene	0.02
Bis-chloromethyl ether	1.0	Pyrene	0.01
1,2-Dichloropropane	0.7	Di-n-butyl phthalate	0.02
Trans-1,3-dichlorproopene	0.4	Benzidine	0.02
Trichloroethylene	0.5	Butyl benzyl phthalate	0.03
Dibromochloromethane	0.3	Chrysene	0.02
Cis-1,3-dichloropropene	0.5	Bis(2-ethylhexyl)phthalate	0.04
1,1,2-Trichloroethane	0.7	Benzo(a)anthracene	0.02
Benzene	0.2	Benzo(b)fluoranthene	0.02
2-Chloroethylvinyl ether	1.0	Benzo(k)fluoranthene	0.02
Bromoform	0.6	Benzo(a)pyrene	0.02
1,1,2,2-Tetrachloroethene	0.9	Indeno(1,2,3-c,d)pyrene	0.02
1,1,2,2-Tetrachloroethane	0.6	Dibenzo(a,h)anthracene	0.02
Toluene	0.1	Benzo(g,h,i)perylene	0.01
Chlorobenzene	0.2	N-nitrosodimethylamine	0.8
Ethylbenzene	0.2	N-nitrosodi-n-propylamine	0.2
		4-Chlorophenyl phenyl ether	0.03
Direct injectables:		3,3'-Dichlorobenzidine	1.0
Acrolein	200	All pesticide and PCB's	1.0
Acrylonitrile	100		

TABLE C9. OTHER ORGANIC COMPOUNDS DETECTED IN THE 16 SAMPLES (µg/ℓ)

Compound	Blank water	Approximate concentration in stream							
		CTHF-1	CTHF-2	CTHF-3	CTHF-4	CTHF-5	CTHF-6	CTHF-7	CTHF-8
Triphenyl phosphine	5	5	0.5		0.5	2			5
Triphenyl phosphine oxide	5	50	10	5	10	10		2	30
α-Terepineol			40	10	30			25	
2-Mercapto benzthiazole				10	20	0.5		10	
1-Cyano-2-benzyloxy ethane					5				
Benzthiazole				30	2		600	40	5
Lauric acid				400			3,000		
Myristic acid							1,000		
Palmitic acid							1,000		
Stearic acid									

Compound	Approximate concentration in stream							
	CTHF-9	CTHF-10	CTHF-11	CTHF-12	CTHF-13	CTHF-14	CTHF-15	CTHF-16
Triphenyl phosphine		5	2	7	10	10	10	10
Triphenyl phosphine oxide	5	5	5	10	30	10	10	5
α-Terepineol		30	20	30	50	50	5	
2-Mercapto benzthiazole						40	30	200
1-Cyano-2-benzyloxy ethane						60	10	100
Benzthiazole	100					200		250
Lauric acid	100							
Myristic acid								
Palmitic acid	400							
Stearic acid	200							

Note.—Blanks indicate compound is below detection limits.

TABLE C10. PRIORITY POLLUTANT METALS ANALYSIS OF DIGESTED SAMPLES, PHENOL, AND CYANIDE
(μg/ℓ)

Species	Detection limit	Concentration in stream							
		CTHF-1	CTHF-2	CTHF-3	CTHF-4	CTHF-5	CTHF-6	CTHF-7	CTHF-8
Priority pollutant metal species:									
Antimony	10	12	30	100	90	132	436	170	146
Arsenic	2	<1	<1	19	<1	1	160	35	5
Beryllium	0.04	-	-	-	-	-	-	-	-
Cadmium	2	5	9	15	15	14	48	16	20
Chromium	4	540	840	640	720	620	1,260	760	800
Copper	4	54	200	90	26	32	760	94	68
Lead	22	168	240	380	250	340	760	400	414
Nickel	36	-	154	132	70	100	480	200	210
Silver	5	-	24	42	26	42	114	62	78
Zinc	1	630	616	520	360	8,600	3,540	460	248
Other species:									
Phenol (total)	1	<1	33	6	12	16	-[a]	4	<1
Cyanide (total)	1	<7	4	<4	72	30	-[a]	<4	<7

(continued)

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[a] Sample arrived at MRC 4 days after sample collection and at room temperature; therefore, no analysis was performed due to poor sample integrity.

TABLE C10 (continued)

Species	Concentration in stream CTHF-9	CTHF-10	CTHF-11	CTHF-12	CTHF-13	CTHF-14	CTHF-15	CTHF-16
Priority pollutant metal species:								
Antimony	380	192	132	116	280	196	160	320
Arsenic	~14	35	15	<1	221	2	<1	9
Beryllium	-	-	-	34	-	-	-	-
Cadmium	120	22	20	48	40	20	34	70
Chromium	1,040	540	760	520	1,000	900	680	1,320
Copper	644	480	46	50	3,060	10,600	100	35,400
Lead	920	520	404	380	700	520	450	1,140
Nickel	468	220	200	62	480	186	220	600
Silver	200	82	68	20	116	70	84	126
Zinc	6,560	7,200	360	140	5,360	6,600	188	12,800
Other species:								
Phenol (total)	13	19	20	18	64	12	3	26
Cyanide (total)	62	<1	<1	<1	8	<4	<4	20

TABLE C11. CONCENTRATION OF OTHER METALS DETECTED BY ICAP ANALYSIS OF SPECIFIC WASTEWATER STREAMS (μg/ℓ)

Metal	Detection limit	Concentration in stream							
		CTHF-1	CTHF-2	CTHF-3	CTHF-4	CTHF-5	CTHF-6	CTHF-7	CTHF-8
Aluminum	12	202	204	800	180	260	3,800	1,470	1,360
Barium	0.2	100	80	84	-	10	580	120	8
Boron	1	610	396	47,200	11,900	8,900	81,000	56,000	31,000
Calcium	0.04	13,720	16,600	16,300	1,050	1,280	114,200	15,600	1,520
Cobalt	6	-	10	22	-	8	72	18	20
Iron	2	1,040	370	520	300	194	2,900	420	300
Magnesium	0.1	5,100	6,720	9,300	240	360	72,000	6,200	408
Manganese	0.5	260	150	360	24	26	2,600	720	26
Molybdenum	10	28	30	52	36	64	160	60	94
Phosphorus	70	260	1,720	4,260	460	860	33,600	5,200	1,470
Silicon	15	13,500	18,200	17,600	2,600	2,600	30,000	21,000	7,600
Sodium	26	59,200	104,000	379,600	13,500	24,800	1,674,000	612,000	244,000
Strontium	0.2	132	160	144	-	-	1,040	140	-
Tin	15	-	-	64	-	-	520	68	-
Titanium	1	6	7	42	8	22	82	28	12
Vanadium	2	28	40	78	14	18	480	64	38

(continued)

TABLE C11 (continued)

Metal	Concentration in stream							
	CTHF-9	CTHF-10	CTHF-11	CTHF-12	CTHF-13	CTHF-14	CTHF-15	CTHF-16
Aluminum	5,000	540	268	212	11,000	1,200	740	3,000
Barium	350	64	-	-	400	196	-	480
Boron	81,000	2,600	500	1,220	4,040	860	740	1,320
Calcium	64,000	16,800	1,060	640	113,600	26,000	920	70,600
Cobalt	52	26	16	44	68	20	20	66
Iron	2,000	428	280	130	1,050	400	180	1,340
Magnesium	28,800	11,400	140	106	81,800	13,000	240	41,200
Manganese	2,860	280	16	40	1,860	700	14	400
Molybdenum	232	98	78	96	154	108	160	400
Phosphorus	24,000	7,600	740	700	49,800	46,800	4,200	141,200
Silicon	22,400	23,800	500	3,360	39,600	12,000	13,300	20,200
Sodium	1,546,000	187,200	13,400	8,600	896,000	530,000	92,000	1,249,000
Strontium	560	146	-	-	1,000	260	-	720
Tin	260	60	-	60	200	50	12	120
Titanium	66	24	12	8	42	16	18	34
Vanadium	240	96	24	10	540	106	30	300

TABLE C12. TRACE METALS QUALITY ASSURANCE RESULTS

Metal	SRM-1643 Standard, µg/ℓ	SRM-1643 Sample value, µg/ℓ	SRM-1643 Percent error	MRC Concentrate Standard, µg/ℓ	MRC Concentrate Sample value, µg/ℓ	MRC Concentrate Percent error	As/Ni dilute Standard, µg/ℓ	As/Ni dilute Sample value, µg/ℓ	As/Ni dilute Percent error	MRC dilute Standard, µg/ℓ	MRC dilute Sample value, µg/ℓ	MRC dilute Percent error
Aluminum	77	66.6	14	–[a]								
Antimony[b]												
Arsenic[b]	76									40	39	3
Barium	18[c]	15.4	14	30,000	33,140	10				1,500	1,421	5
Beryllium[b]	19	16.4	14									
Boron												
Cadmium[b]	8	7.0	12	4,000	4,080	2				200	205	2
Calcium	27,000[d]	26,900	0.3									
Chromium[b]	15	16.9	13	10,000	10,040	0.4				500	502	0.1
Cobalt	17	19.0	12									
Copper[b]	16	18.5	16	5,000	5,055	1				250	253	1
Iron	75	73.0	3	30,000	29,230	3				1,500	1,473	2
Lead[b]	20	8.5	57	50,000	50,870	2				2,500	2,523	0.9
Magnesium	7,000[d]	6,140	12									
Manganese	29	27.3	6	20,000	20,790	4				1,000	1,001	0.1
Molybdenum	105	95.2	9									
Nickel[b]	49	66.1	35				250	236	5.6			
Phosphorus												
Silicon												
Silver[b]	3.4	–[e]					250	269	7.6			
Sodium	10,000[d]	11,500	15									
Strontium	212	244	15									
Tin												
Titanium												
Vanadium	50	87.2	74									
Zinc[b]	65	61.6	5	10,000	10,360	4				500	514	2.8

Metal	CRRP-8 6230	CRRP-8 6239	CRRP-8 % diff.	CRRP-16 6238	CRRP-16 6240	CRRP-16 % diff.	Blank HNO_3	Blank RO water
Aluminum	1,300	1,400	6	3,000	3,000	0	130	165
Antimony	146	200	31	330	390	17	88	92
Arsenic								
Barium	8	–	–	400	400	0	3	2
Beryllium	–	–	–	–	–	–	0.3	0.6
Boron	31,000	31,800	3	1,320	1,360	3	160	57
Cadmium	20	28	33	70	70	0	12	13
Calcium	1,520	1,880	21	70,600	70,800	0.3	680	511
Chromium	800	820	2	1,320	1,350	2	740	370
Cobalt	20	22	10	66	74	11	7	12
Copper	68	90	28	35,400	35,400	0	28	25
Iron	300	320	6	1,340	1,360	1	145	92
Lead	414	500	19	1,140	1,250	9	286	260
Magnesium	420	450	7	41,200	41,600	1	75	50
Manganese	26	26	0	400	406	1	6	5
Molybdenum	94	122	26	400	416	4	54	51
Nickel	210	260	21	600	650	8	82	114
Phosphorus	1,470	1,980	30	141,200	143,000	1	500	480
Silicon	7,600	8,080	6	20,200	23,400	15	350	480
Silver	78	100	25	126	134	6	32	51
Sodium	264,000	282,400	7	1,349,000	1,345,200	0.3	3,380	1,440
Strontium	–	12	–	720	720	0	3	3
Tin	–	43	–	120	136	12	4	–
Titanium	12	16	29	34	54	45	7	<7
Vanadium	38	42	10	300	300	0	11	14
Zinc	248	320	25	12,800	21,600	51	350	690

[a] Blanks indicate no metal standard included.

[b] Priority pollutant metal.

[c] Barium is not certified because of the large difference between its initial concentration (39 ng/g), corresponding to the amount added to the water, and the stabilized concentration (18 ng/g). However, this stabilized concentration has remained constant in the test-bottles for over four months.

[d] SRM 1643 approximates the elemental composition of fresh water - 27 µg/g calcium, 10 µg/g sodium, 7 µg/g magnesium, and 2 µg/g potassium.

[e] Metal standard concentration is below detection limit.

For quality assurance standard solutions in two concentration ranges of phenol and cyanide were prepared according to Reference 4 procedures. A blind repeat of each standard was also included. Results of the analyses are shown in Table 13.

TABLE C13. QUALITY CONTROL SAMPLES FOR PHENOL (TOTAL) AND CYANIDE (TOTAL)

Compound	Standard value, mg/ℓ	Sample value, mg/ℓ	Percent error
Phenol (total)	10	11	10
	10	15	50
	300	300	0
	300	270	10
Cyanide (total)	10	4	60
	10	4	60
	300	384	28
	300	348	16

SECTION 5

BIOASSAY TESTS

MICROBIAL MUTAGENICITY (AMES TEST)

Test Procedure

The purpose of the mutagenicity bioassay (Ames test) was to determine if a chemical mutagen (possibly a carcinogen) was present in the 14 samples tested. The plant intake water (CTHF-1) and hyperfiltration rinse water (CTHF-2) were not tested in any of the bioassays.

To date the most sensitive assay for deoxyribonucleic acid (DNA) damage is the induction of mutations in bacteria. The Ames test, the most developed of the bacterial mutagenesis tests, used mutant strains of *Salmonella typhimurium* which were specially selected because of their abilities to detect specific types of mutations. The assay procedure with *S. typhimurium* has proven to be 85% to 90% accurate in detecting mutagens, and it has about the same accuracy in identifying chemicals that are not carcinogenic (6). For example, the TA1535 strain was designed to detect mutations due to base-pair substitutions. This strain responded particularly well to alkylating agents. Similarly, the TA1537 and TA1538 strains were used to detect frameshift mutations. Tester strains also included mutations which greatly increase their overall sensitivity to mutagens. One of these was responsible for loss of the DNA excision repair system, while the other was responsible for loss of the lipopolysaccharide barrier that coats the surface of the bacteria, thereby enhancing the penetration of large molecules.

Mutant *S. typhimurium* tester strains lack the ability to synthesize histidine and are therefore unable to grow unless histidine is supplied. These bacteria are cultured in media containing minimal levels of histidine to sustain growth. Under these conditions only microscopic colonies of bacteria develop during the course of the test. However, if a mutagen is added to the medium, a reversion occurs in a certain number of the bacteria, restoring their ability to synthesize histidine. This reversion (back-mutation) is evidenced by the appearance of visible colonies in the histidine-limited agar, thus indicating the presence of a chemical mutagen.

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(6) McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. Detection of Carcinogens as Mutagens in the *Salmonella*/Microsome Test: Assay of 300 Chemicals. Proceedings of the National Academy of Science, 72:5135-5139, 1975.

Many compounds are not directly acting mutagen but are converted to active forms by normal body metabolism. A special microsomal preparation (usually liver) is added to the *S. typhimurium* tests to simulate *in vivo* metabolic actions. In practice, the substance is tested with and without this microsomal preparation to determine whether it requires metabolic transformation or is, itself, mutagenically active.

The 14 samples were tested following the procedure of Ames described in Reference 7. An outline of the procedure used by MRC is given in Table 14. MRC currently purchases the S-9 fraction from Litton Bionetics. In addition to the details of the procedure the following steps were taken. All samples were stored at 4°C until analyzed. Each sample was filter sterilized then passed through three filters (1.2 µm, 0.45 µm, and 0.22 µm) in series. Each filtrate was then tested in duplicate, with and without microsome addition. Dose response tests were conducted by adding the following amounts of sample per plate, 10 µℓ, 50 µℓ, 100 µℓ, 500 µℓ, and 1,000 µℓ. Several samples were retested because of poor growth or the possibility of an increased response.

Results of the Ames Tests

None of the 14 samples were mutagenic in the Ames *S. typhimurium* mutagenicity test under the conditions tested. The criteria used to evaluate the samples were that the sample must increase the number of revertants by a factor of two over the spontaneous revertants of the controls and exhibit a dose response with the increase plate dosage. Toxicity was observed by a sparse lawn at the highest concentrations (1,000 µℓ/plate) for samples CTHF-3, 4, 5, 9, 14, and 15. Sample CTHF-6 could not readily be filter sterilized and when streaked on nutrient agar showed bacterial contamination. Therefore, this sample was not tested by the Ames test due to the quantity of sample needed. Table 15 summarizes the data. The actual test data including positive controls and background controls are listed in Appendix B.

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(7) Ames, B. N., J. McCann, and E. Yamasaki. Methods for the Detection of Carcinogens and Mutagens with the *Salmonella*/Mammalian-Microsome Mutagenicity Test. Mutagenicity Research, 31:347-364, 1975.

TABLE C14. PROCEDURE FOR AMES MUTAGENICITY TEST

Tester strains: TA98, TA100, TA1535, TA1537, TA1538, TA92, HisG46
Strains are stored frozen in nutrient broth at -80°C
Strains are routinely checked for their histidine requirements, rfa$^-$ deletion, urvB$^-$ deletion, plasmids, and spontaneous revertants.

A. S-9 Mix Preparation:

Reagent	Stock solution	mℓ/S-9 mixture	Storage
$MgCl_2$/KCl	0.4M/1.65M	0.2 mℓ	refrigerate
G-6-P	0.5M	100 μℓ	frozen
Na Phosphate	0.2M (pH 7.4)	5.0 mℓ	refrigerate
NADP	0.1M	0.4 mℓ	frozen
Sterile H_2O		3.3 mℓ	--
S-9 fraction (thawed, kept on ice)		1.0 mℓ	frozen

B. Preparation of Top and Bottom Agar:

1. Top Agar - 0.6% Agar (6 g/ℓ)
   0.5% NaCl (5 g/ℓ)

   Autoclaved, stored in sterile bottles (50 mℓ and 100 mℓ aliquots)

   Top is melted in autoclave or steam bath before use.

   0.5 mM L-Histidine • HCl/0.5 mM Biotin solution is added to the melted top agar just before each test.

2. Bottom Agar

	g/ℓ
$MgSO_4 \cdot 7H_2O$	0.2
Citric Acid H_2O	2.0
K_2HPO_4 (anhydrous)	10.0
$NaNH_4HPO_4 \cdot 4H_2O$	3.5
Agar	15.0
Glucose	20.0

C. Preparation of Test Substance:

A compound is dissolved in sterile H_2O, DMSO, ethanol or *p*-dioxane. Up to 100 μℓ ethanol or *p*-dioxane can be used per plate.

D. Assay:

1. 2 mℓ molten top agar (45°C) (with histidine and biotin)
2. 0.1 mℓ bacteria
3. 1-100 μℓ test compound
4. 0.5 mℓ S-9 mix (when added)

Plates are incubated at 37°C, in dark, 48 hr. Revertants are scored for test compounds and controls.

TABLE C15. RESULTS OF AMES MICROBIAL MUTAGENICITY TESTS

Sample number	Result	Amount tested per plate, µℓ
CTHF-3	Negative	10 to 1,000
CTHF-4	Negative	10 to 1,000
CTHF-5	Negative	10 to 1,000
CTHF-7	Negative	10 to 1,000
CTHF-8	Negative	10 to 1,000
CTHF-9	Negative	10 to 1,000
CTHF-10	Negative	10 to 1,000
CTHF-11	Negative	10 to 1,000
CTHF-12	Negative	10 to 1,000
CTHF-13	Negative	10 to 1,000
CTHF-14	Negative	10 to 1,000
CTHF-15	Negative	10 to 1,000
CTHF-16	Negative	10 to 1,000

CYTOTOXICITY TEST

Test Procedure

MRC performed clonal assay acute toxicity tests on 14 samples using Chinese hamster ovary cells (CHO-Kl). The purpose of cytotoxicity tests was to measure metabolic impairment and death in mammalian cells due to exposure to the wastewater samples. These primary cell cultures have some degree of metabolic repair capability.

Samples were tested according to the procedure described in References 8 and 9 and shown in Table 16.

In general, the test procedure involves trysinizing stock cultures of CHO-Kl cells and counting. A cell dilution was made with media to concentration of 60 cells/mℓ. Five milliliters (∿300 cells) of this dilute cell suspension were added to a T-25

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(8) Malcolm, A. R., B. H. Pringle, and H. W. Fisher. Chemical Toxicity Studies with Cultured Mammalian Cells. In: Bioassay Techniques and Environmental Chemistry, G. E. Glass, ed. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1974. pp. 217-230.

(9) Wininger, M. T., F. A. Kulik, and W. D. Ross. *In Vitro* Clonal Cytotoxicity Assay Using Chinese Hamster Ovary Cells (CHO-Kl) for Testing Environmental Chemicals. *In Vitro*, 14;381, 1978.

TABLE C16. PROCEDURE FOR CHO-K1 CLONAL CYTOTOXICITY TEST

The details of the CHO-K1 clonal toxicity test are as follows:

Cell line:	Chinese hamster ovary epithelial cells ATCC #CCL 61
Medium:	F-12 GIBCO #H-17 10.8 g/ℓ $NaHCO_3$ 1.18 g/ℓ 10% fetal calf serum, virus, mycoplasma screened GIBCO #629
Incubation:	37°C, 5% CO_2, saturated humidity
Samples:	6 controls (blank) 5-7 concentrations of test compound in triplicate

Test Procedure:

- To stock CHO-K1, add 5 mℓ 0.25% Trypsin at 37°C for 5 min to 10 min
- Shake cells and add to centrifuge tube
- Add 5 mℓ media to flask, shake and add to centrifuge tube
- Centrifuge 5 min at 500 g, pour off liquid, retaining cells
- Add 10 mℓ medium, shake, centrifuge 5 min, pour off medium
- Add 10 mℓ medium, shake
- Make hemocytometer count of trypsinized cells
- Dilute so that 5 mℓ media contains 300 to 500 cells
- Add 5 mℓ media and cells to T-25 flasks
- Incubate 12 to 18 hours to allow attachment using normal media
- Replace with 5 mℓ of premixed media and sample
- Incubate 6 to 7 days total
- Fix with 10% formaldehyde/0.5% NaCl/4% methanol for 30 min
- Stain with crystal violet (0.04% for 15 min)
- Count colonies of remaining cells macroscopically using Fisher Count-All Model 600
- Score with respect to experimental versus controls at % survival

flask. Cultures were incubated for 18 hr to permit cell attachment using normal media. All wastewater samples were stored at 4°C and filter sterilized through a series of 1.2 μm, 0.45 μm, and 0.22 μm filters. Filtrates were then applied to the plates 18 hr after seeding the plates. These plates were incubated at 37°C in a carbon dioxide incubator for 5 days.

At the end of this time period, the cells were fixed with 10% formaldehyde, 0.5% sodium chloride, and 4% methanol for 30 min followed by staining with 0.04% crystal violet for 5 min. Stained colonies were then counted. The percent colony formation was calculated by comparing the control plates with the wastewater sample containing plates. EC_{50} (effective concentration at which 50% of the cells show metabolic impairment) determinations were calculated from dose response curves.

The concentrations used for these samples were 0.2 µℓ, 2.0 µℓ, 10 µℓ, 50 µℓ, 100 µℓ, 150 µℓ, and 200 µℓ of sample per milliliter of media. All samples were tested in triplicate at each concentration and retests were performed on those samples that exhibited toxicity.

Cytotoxicity Results

Results of the CHO cytotoxicity test are shown in Table 17. The raw data collected in the tests are given in Appendix C.

TABLE C17. RESULTS OF THE CHO-K1 CYTOTOXICITY TEST

Sample	Results	Concentration range tested, µℓ/mℓ media
CTHF-3	No acute toxicity	0.2 to 200
CTHF-4	No acute toxicity	0.2 to 200
CTHF-5	No acute toxicity	0.2 to 200
CTHF-6	EC_{50} = 90 µℓ/mℓ media = 9% solution	0.2 to 200
CTHF-7	No acute toxicity	0.2 to 200
CTHF-8	No acute toxicity	0.2 to 200
CTHF-9	No acute toxicity	0.2 to 200
CTHF-10	No acute toxicity	0.2 to 200
CTHF-11	No acute toxicity	0.2 to 200
CTHF-12	No acute toxicity	0.2 to 200
CTHF-13	EC_{50} = 100 µℓ/mℓ media = 10% solution	0.2 to 200
CTHF-14	No acute toxicity	0.2 to 200
CTHF-15	No acute toxicity	0.2 to 200
CTHF-16	EC_{50} > 200 µℓ/mℓ media > 20% solution	0.2 to 200

At the concentration range tested (0.2 µℓ/mℓ to 200 µℓ/mℓ) toxicity was exhibited with the CTHF-6, 13, and 16 samples, while all other samples were nontoxic. Toxicity is reported as EC_{50} (effective concentration at which 50% of the cells shown metabolic impairment). EC_{50} values for samples CTHF-6 and CTHF-13 are 90 µℓ/mℓ and 100 µℓ/mℓ, respectively. Expressed in different terms, a wastewater concentration of 9% for CTHF-6 and 13% for CTHF-13 would metabolically impair 50% of the CHO cells. Sample CTHF-16 showed toxic effects only at the highest concentration of 200 µℓ/mℓ, thus no EC_{50} could be calculated and is expressed as EC_{50} >200 µℓ/mℓ or EC_{50} >20%.

Cadmium chloride was used as a positive control and had an average EC_{50} of 0.15 µℓ/mℓ or 0.015% solution.

ACUTE STATIC BIOASSAYS WITH FATHEAD MINNOWS AND DAPHNIDS

Fourteen of the sixteen wastewater samples (excluding CTHF-1 and 2) were collected by Clemson University in 5-gal plastic containers for subsequent bioassay analysis with fathead minnows (*Pimephales promelas*) and daphnids (*Daphnia magna*). Clemson University sent the samples directly to the laboratory designated by MRC: EG&G Bionomics Marine Research Laboratory, Wareham, Massachusetts. The following subsections describe the test procedures and materials used and results of the tests.

Fathead Minnow Test Procedures

Procedures used in this 96-hr, static acute toxicity test followed those described in Reference 10. Wastewater samples were delivered by Clemson University, Clemson, South Carolina, in June 1978, in 5-gal. plastic containers. A characterization of each sample is presented in Appendix D.

The fathead minnows (*Pimephales promelas*) used in the determinations were obtained by EG&G from a commercial fish supplier in Missouri. These fish were assigned a lot number and held in 500-ℓ fiberglass tanks. Well water characterized as having total hardness and alkalinity, as calcium carbonate, ranged from 28 mg/ℓ to 44 mg/ℓ and 20 mg/ℓ to 30 mg/ℓ, respectively (4), a pH range of 6.7 to 7.4, a temperature of 22 ± 1°C and a specific conductance range of 95 to 170 micromhos per centimeter flowed through the tank at a minimum of 4 ℓ/min. The specific conductance was measured with a Model #33 YSI conductivity meter. Experimental animals were maintained under these conditions for a minimum of 14 days. During this time period, all fish were fed a dry pelleted food, *ad libitum*, daily and ground liver weekly, except during the 48 hours prior to testing. Mortality observed during this 2-day period ranged from 0.16% to 0.80%.

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(10) Peltier, W. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. EPA-600/4-78-012 (PB 276 690) U.S. Environmental Protection Agency, Cincinnati, Ohio, January 1978. 63 pp.

Toxicity tests were conducted in 19.6-ℓ glass jars which contained 15 ℓ of test solution. The diluent water used was soft water reconstituted according to recommended procedures (10). A characterization of this water also appears in Appendix D. Wastewater samples were mixed with diluent water to provide the appropriate percentage concentrations. A control jar containing the same dilution water and maintained under the same conditions as test concentrations, but containing no wastewater sample, was established. Test solution temperatures were controlled by a system designed to maintain test temperatures at 22 ± 1°C. Test solutions were not aerated, except where noted.

Ten fathead minnow with a mean and range (N=30) net weight of 0.53 g (0.21 g to 1.1 g) and a total length and range of 40 mm (31 mm to 52 mm) were randomly distributed to each test jar within 3 hours after the test solutions were mixed. This time period was necessary to warm the solutions to 22 ± 1°C.

During toxicity determinations, pH and dissolved oxygen concentrations of test solutions were measured at 0-, 24-, 48-, 72-, and 96-hr in the control, high, middle and low test concentrations. Temperatures were also measured in the control jars at the above-mentioned time intervals. Specific conductance, total hardness and alkalinity were measured in the control, high, middle and low test concentrations at 0-hr. The pH was measured with a Model #175 Instrumentation Laboratory pH meter and combination electrode and the temperature and dissolved oxygen (DO) with a Model #57 YSI combination oxygen-temperature meter and probe.

Test concentrations and corresponding percentage mortality data derived from each test were used to calculate 24-, 48-, 72-, and 96-hr median lethal concentrations (LC_{50}'s) and 95% confidence intervals by means of the moving average angle method (11). The LC_{50} is defined as the calculated nominal concentration of the wastewater sample in diluent water which caused 50% mortality in the fathead minnow population at the stated exposure interval.

Prior to analysis by this method, nominal concentrations were transformed to logarithms and corresponding percentage mortalities to angles. Each group of three successive angles was then averaged and the LC_{50} was estimated by linear interpolation between the successive concentrations whose average angles bracketed 45°.

Daphnid Test Procedure

Daphnia magna (<24 hr old) used in this 48-hr acute toxicity test were from laboratory stocks cultured at EG&G, Bionomics.

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(11) Harris, E. K. Confidence Limits for the LD_{50} Using the Moving Average Angle Method. Biometrics, 4(3):157-164, 1959.

Deionized, reconstituted well water with a total hardness of 200 mg/ℓ as $CaCO_3$, a pH of 8.1, a temperature of 22 ± 1°C and a dissolved oxygen (DO) concentration of greater than 60% of saturation was used to culture these animals. A description of each of the 14 samples is given in Appendix D.

Procedures used in this acute toxicity test were based on protocols in Reference 12 except where stated otherwise.

Two independent tests involving two different series of sample concentrations were performed in this study. A preliminary (range-finding) test was performed to define a narrower range of concentrations to be used in a subsequent definitive test. Mortality data derived from the definitive test were used to calculate a median lethan concentration (LC_{50}) and its 95% confidence limit utilizing the moving average method (13). The LC_{50} is the calculated nominal concentration of the wastewater sample in diluent water which produces 50% mortality in the daphnid population at the stated times of exposure.

Static toxicity tests were conducted in 250-mℓ beakers which contained 150 mℓ of test solution. Diluent water used in this study had the same water quality characteristics as described in Appendix D. For each test concentration, the appropriate amount of the wastewater sample was introduced into the required volume of diluent water to total 750 mℓ and mixed with a magnetic stirrer. This solution was then divided into three 150-mℓ aliquots in triplicate beakers to provide replicate exposure treatments. The remaining 300 mℓ were used for 0-hr DO, pH, specific conductance, alkalinity, and total hardness determinations.

A control, consisting of the same dilution water and conditions, but with no effluent, was established. All test vessels were maintained at 22 ± 1°C and test solutions were not aerated during the test. Five daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 15 daphnids per concentration.

During the tests, the dissolved oxygen concentration, pH, and temperature of test solutions were monitored at the initiation and termination of the toxicity test in the control, high, middle and low test concentrations. Total hardness, specific conductance and alkalinity were monitored at the initiation of the study in the control, high, middle and low test concentrations. DO and

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(12) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009 (PB 242 105) U.S. Environmental Protection Agency, Duluth, Minnesota, March 1975. 61 pp.

(13) Personal communication with C. E. Stephan, U.S. Environmental Protection Agency, Duluth, Minnesota, 1978.

temperature were measured with a USI dissolved oxygen meter and combination oxygen-temperature probe. pH was measured with an Instrumentation Laboratory pH meter. The total hardness determinations of diluent water were conducted according to Reference 4. Salinity and specific conductance were determined with an American Optics refractometer and a YSI conductivity bridge, respectively.

Results of Fathead Minnow and Daphnia Bioassay

Appendix E shows the raw data as reported by EG&G Bionomics for each of the 14 fathead minnow bioassay samples. The first table for each sample shows the pH, DO, specific conductance, total hardness and alkalinity measurements made during the 96-hr test. The second table associated with each sample shows the percent mortality raw data used to calculate LC_{50} values.

Appendix F shows the raw data as reported by EG&G Bionomics associated with each sample for the daphnia acute toxicity tests. The first table for each sample shows the water quality analyses of each as a function of concentration tested. The second table shows the raw mortality data used to calculate LC_{50} values.

Results of the two static acute toxicity tests are shown in Table 18. In addition to LC_{50} values, the mortality data was used to extrapolate the concentration of wastewater sample which would produce no discernible effect on the fathead minnows or daphnids. Analysis of the data indicate the four permeate samples (CTHR-5, 8, 12, and 15) produced no or very little mortality to fathead minnows. The most toxic wastewater samples were the concentrates (CTHF-6, 9, 13, and 16).

RANGE-FINDING ACUTE TOXICITY 14-DAY RAT TEST

The major objective of any biological testing procedure is the identification of toxicological problems at minimal cost. Therefore, a two-step approach was used to evaluate the acute *in vivo* toxicity of samples containing unknown compounds. The first approach was based on the quantal (all-or-none) response; the second was based on the quantitative (graded) response. The quantal test was used to determine whether or not the quantitative assay was necessary. Fourteen wastewater samples were shipped by Clemson University to the MRC designated laboratory: Litton Bionetics, Kensington, Maryland. The following sections describe the test procedures used and bioassay results.

The Quantal Test Procedure

Five male and five female young adult rats (weighing approximately 250 g each) were purchased by Litton Bionetics from the supplier and conditioned at the laboratory for a minimum of 5 days. A single 10 mℓ/kg dose of undiluted sample was administered by gavage to each animal. Immediately following administration of

TABLE C18. RESULTS OF STATIC ACUTE TOXICITY TESTS TO FATHEAD MINNOWS AND DAPHNIDS

	Fathead minnow acute toxicity					Daphnia acute toxicity		
	LC$_{50}$ at time intervals, % sample solution				No discernible effect concentration at 96-hr, %	LD$_{50}$ at time intervals, % sample solution		No discernible effect concentration at 48-hr, %
Sample	24-hr	48-hr	72-hr	96-hr		24-hr	48-hr	
CTHF-3	>11 <24[a]	16 (13 to 21)[b]	16 (13 to 21)	16 (13 to 21)	0.53	59 (49 to 72)	26 (20 to 34)	<13
CTHF-4	28 (24 to 33)	28 (24 to 33)	28 (24 to 33)	28 (24 to 33)	4.6	>60 <100	53 (45 to 62)	22
CTHF-5	NAT[c]	NAT	NAT	-[d]	-[e]	60 (36 to 100)	42 (35 to 51)	13
CTHF-6	>2.4 <5.3	1.6 (1.2 to 2.3)	1.6 (1.2 to 2.3)	1.5 (1.0 to 2.2)	0.24	10 (7.3 to 15)	5.1 (4.2 to 6.2)	2.8
CTHF-7	16 (7.8 to 32)	13 (7.8 to 22)	13 (7.8 to 22)	13 (7.8 to 22)	<7.8	>100	25 (20 to 31)	<13
CTHF-8	NAT	NAT	NAT	NAT	-[e]	>100	>100	60
CTHF-9	>3.2 <5.3	2.7 (2.2 to 3.3)	2.2 (1.6 to 2.9)	2.0 (1.5 to 2.8)	<0.41	13 (11 to 16)	9.9 (8.3 to 12)	4.1
CTHF-10	17 (14 to 21)	10 (8.1 to 12)	10 (8.1 to 12)	9.7 (7.5 to 12)	<4.6	>100	33.5 (27.6 to 50.4)	22
CTHF-11	>100	>100	87 (20 to 100)	82 (21 to 100)	36	>100	>60 <100	60
CTHF-12	NAT	NAT	NAT	NAT	-[e]	>60 <100	>60 <100	60
CTHF-13	21 (1.6 to 27)	1.7 (1.2 to 2.1)	1.7 (1.2 to 2.1)	1.6 (1.2 to 2.0)	0.78	11 (2.5 to 48)	4.1 (3.4 to 4.9)	2.8
CTHF-14	41 (35 to 48)	41 (35 to 48)	25 (21 to 29)	25 (21 to 29)	11	>60 <100	49 (41 to 58)	<13
CTHF-15	NAT	NAT	NAT	NAT	-[e]	>100	80 (71 to 90)	<13
CTHF-16	9.7 (6.3 to 14)	5.3 (4.1 to 6.8)	5.3 (4.1 to 6.8)	5.3 (4.1 to 6.8)	<0.36	>100	17 (13 to 23)	2.8

[a] >11 <24 = 24-hr LC$_{50}$ value is greater than 11% but less than 24% solution of the wastewater.

[b] Values in parentheses are 95% confidence interval.

[c] No acute toxicity.

[d] Only 30% mortality occurred in 100% solution of wastewater.

[e] Not calculated due to insufficient mortality.

the test substance and at frequent intervals during the first day, observations were recorded on all toxic signs or pharmacological effects as described in Table 19 (14). The frequency and severity of the signs were scored. Particular attention was paid to time of onset and disappearance of signs. Daily observations were made on all animals through a 14-day observation period. Wastewater samples which produced harmful effects *in vivo* and did not result in deaths were further investigated. At termination of the observation period, all surviving animals were killed and necropsies performed. Similarly, necropsies were performed on all animals that died during the course of the study.

If mortality did not occur in the quantal study, no further work was done on the test substance, and the LD_{50} was reported as greater than 10 g/kg.

The Quantitative Assay Procedure

If a single animal in the quantal study died in the 14-day observation period, a quantitative study was performed. Eighty animals equally divided by sex were maintained for 7 days in quarantine to determine good health in the study population. From these, 40 animals then were randomly divided into 4 groups of 5 male and 5 female animals per group. The test substance, treated as in the quantal test, was administered in graded dosages according to the following schedule: 3.0 g/kg, 1.0 g/kg, 0.3 g/kg, and 0.1 g/kg. Dosage was related to the numbers of animals that died and to the severities and types of signs. Observations, duration of study, and necropsies were carried out as indicated above. The LD_{50} was calculated by the method described in Reference 14.

The range-finding tests were conducted at Litton Bionetics under the direction of Dr. R. Beliles. Actual experimental design parameters used in this study were as follows. Young adult rats of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. Animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hr light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from cages.

Wastewater samples were kept refrigerated until used. A single undiluted dose of 10 mℓ/kg of test material was administered by

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(14) Duke, K. M., M. E. Davis, and A. J. Dennis. IERL-RTP Procedures Manual: Level I Environmental Assessment Biological Tests for Pilot Studies. EPA-600/7-77-043 (PB 268 484) U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, April 1977. 114 pp.

TABLE C19. PHYSICAL EXAMINATIONS IN ACUTE TOXICITY TESTS IN RODENTS (3)

Organ system	Observation and examination	Common signs of toxicity
Central nervous system and somatomotor	Behavior	Change in attitude to observer, unusual vocalization, restlessness, sedation.
	Movements	Twitch, tremor, ataxia, catatonia, paralysis, convulsion, forced movements.
	Reactivity to various stimuli	Irritability, passivity, anaesthesia, hyperanaesthesia.
	Cerebral and spinal reflexes	Sluggishness, absence.
	Muscle tone	Rigidity, flaccidity.
Autonomic nervous system	Pupil size	Myosis, mydriasis.
	Secretion	Salivation, lacrimation.
Respiratory	Nostrils	Discharge.
	Character and rate of breathing	Bradypnoea, dyspnoea, Cheyne-Stokes breathing, Kussmaul breathing.
Cardiovascular	Palpataion of cardiac region	Thrill, bradycardia, arrhythmia, stronger or weaker beat.
Gastrointestinal	Events	Diarrhea, constipation.
	Abdominal shape	Flatulence, contraction.
	Feces consistency and color	Unformed, black or clay colored.
Genitourinary	Vulva, mammary glands	Swelling.
	Penis	Prolapse.
	Perineal region	Soiled.
Skin and fur	Color, turgor, integrity	Reddening, flaccid skinfold, eruptions, piloerection.
Mucous membranes	Conjunctiva, mouth	Discharge, congestion, hemorrhage cyanosis, jaundice.
Eye	Eyeball	Exophthalmus, nystagmus.
	Transparency	Opacities.
Others	Rectal or pay skin temperature	Subnormal, increased.
	Injection site	Swelling.
	General condition	Abnormal posture, emaciation.

gastric intubation to five rats of each sex. If any rats died at this dose, an LD_{50} value was to be determined by giving additional doses of the test material.

The rats were observed frequently on the day of treatment and daily thereafter. Animals were weighed on the day of treatment, and on days 7 and 14 following treatment. All surviving animals were killed 14 days after treatment and necropsies were performed.

Results of Rat Acute Toxicity Test

Final reports from Litton Bionetics by Dr. R. Beliles state that no rats died as a result of single maximum dosages of the 14 wastewater samples. Necropsy results indicated no sample related effects were observed. Therefore, no samples were subjected to quantitative analysis.

REFERENCES

1. Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. Draft final report, U.S. Environmental Protection Agency, Cincinnati, Ohio, April 1977. 145 pp.

2. Rawlings, G. D. Source Assessment: Textile Plant Wastewater Toxics Study - Phase I. EPA-600/2-78-004h, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, March 1978. 166 pp.

3. Manual of Methods for Chemical Analysis of Water and Wastes. EPA-625/6-76-003a (PB 259 973) U.S. Environmental Protection Agency, Cincinnati, Ohio, 1976. 317 pp.

4. Standard Methods for the Examination of Water and Wastewater, Fourteenth Edition. American Public Health Association, Washington, D.C., 1976. 874 pp.

5. Carter, M. J., and M. T. Huston. Preservation of Phenolic Compounds in Wastewaters. Environmental Science and Technology, 12(3):309-313, 1978.

6. McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. Detection of Carcinogens as Mutagens in the *Salmonella*/Microsome Test: Assay of 300 Chemicals. Proceedings of the National Academy of Science, 72:5135-5139, 1975.

7. Ames, B. N., J. McCann, and E. Yamasaki. Methods for the Detection of Carcinogens and Mutagens with the *Salmonella*/Mammalian-Microsome Mutagenicity Test. Mutagenicity Research, 31:347-364, 1975.

8. Malcolm, A. R., B. H. Pringle, and H. W. Fisher. Chemical Toxicity Studies with Cultured Mammalian Cells. In: Bioassay Techniques and Environmental Chemistry, G. E. Glass, ed. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1974. pp. 217-230.

9. Wininger, M. T., F. A. Kulik, and W. D. Ross. *In Vitro* Clonal Cytotoxicity Assay Using Chinese Hamster Ovary Cells (CHO-Kl) for Testing Environmental Chemicals. *In Vitro*, 14:381, 1978.

10. Peltier, W. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. EPA-600/4-78-012 (PB 276 690) U.S. Environmental Protection Agency, Cincinnati, Ohio, January 1978. 63 pp.

11. Harris, E. K. Confidence Limits for the LD$_{50}$ Using the Moving Average Angle Method. Biometrics, 4(3):157-164, 1959.

12. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009 (PB 242 105) U.S. Environmental Protection Agency, Duluth, Minnesota, March 1975. 61 pp.

13. Personal communication with C. E. Stephan, U.S. Environmental Protection Agency, Duluth, Minnesota, 1978.

14. Duke, K. M., M. E. Davis, and A. J. Dennis. IERL-RTP Procedures Manual: Level I Environmental Assessment Biological Tests for Pilot Studies. EPA-600/7-77-043 (PB 268 484) U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, April 1977. 114 pp.

15. Standard for Metric Practice. ANSI/ASTM Designation: E 380-76$^{\epsilon}$, IEEE Std. 268-1976, American Society for Testing and Materials, Philadelphia, Pennsylvania, February 1976. 37 pp.

APPENDIX CA

PRIORITY POLLUTANT ANALYSIS FRACTIONS

TABLE CA1. VOLATILE COMPOUNDS

Compound	Compound
Chloromethane	1,2-Dichloropropane
Dichlorodifluoromethane	*trans*-1,3-dichloropropene
Bromomethane	Trichloroethylene
Vinyl chloride	Dibromochloromethane
Chloroethane	*Cis*-1,3-dichloropropene
Methylene chloride	1,1,2-Trichloroethane
Trichlorofluoromethane	Benzene
1,1,-Dichloroethylene	2-Chloroethyl vinyl ether
1,1-Dichloroethane	Bromoform
trans-1,2,-dichloroethane	1,1,2,2-Tetrachloroethylene
Chloroform	1,1,2,2-Tetrachloroethane
1,2-Dichloroethane	Toluene
1,1,1-Trichloroethane	Chlorobenzene
Carbon tetrachloride	Ethylbenzene
Bromodichloromethane	Acrolein
Bis(chloromethyl) ether	Acrylonitrile

TABLE CA2. BASE NEUTRAL EXTRACTABLE COMPOUNDS

Compound	Compound
1,3-Dichlorobenzene	Anthracene
1,4-Dichlorobenzene	Diethyl phthalate
Hexachloroethane	Dimethyl phthalate
1,2-Dichlorobenzene	Fluoranthene
Bis(2-chloroisopropyl) ether	Pyrene
Hexachlorobutadiene	Di-n-butyl phthalate
1,2,4-Trichlorobenzene	Benzidine
Naphthalene	Butyl benzyl phthalate
Bis(2-chloroethyl) ether	Chrysene
Hexachlorocyclopentadiene	Bis(2-ethylhexyl) phthalate
Nitrobenzene	Benz(a)anthracene
Bis(2-chloroethoxy) methane	Benzo(b)fluoranthene
2-Chloronaphthalene	Benzo(k)fluoranthene
Acenaphthylene	Benzo(a)pyrene
Acenaphthene	Indeno(1,2,3-cd)pyrene
Isophorone	Dibenz(a,h)anthracene
Fluorene	Benzo(g,h,i)perylene
2,6-Dinitrotoluene	N-nitrosodimethylamine
1,2-Diphenylhydrazine	N-nitroso-di-n-propylamine
2,4-Dinitrotoluene	4-Chlorophenyl phenyl ether
N-nitrosodiphenylamine	3,3'-Dichlorobenzidine
Hexachlorobenzene	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin[a]
4-Bromophenyl phenyl ether	
Phenanthrene	Bis-(chloromethyl) ether

[a] This compound was specifically listed in the consent decree. Because of TCDD's extreme toxicity, EPA recommends that laboratories not acquire analytical standards for this compound.

TABLE CA3. ACID EXTRACTABLE COMPOUNDS

2-Chlorophenol
Phenol
2,4-Dichlorophenol
2-Nitrophenol
p-Chloro-*m*-cresol
2,4,6-Trichlorophenol
2,4-Dimethylphenol
2,4-Dinitrophenol
4,6-Dinitro-*o*-cresol
4-Nitrophenol
Pentachlorophenol

TABLE CA4. PESTICIDES AND PCB

Compound
β-Endosulfan
α-BHC
γ-BHC
β-BHC
Aldrin
Heptachlor
Heptachlor epoxide
α-Endosulfan
Dieldrin
4,4'-DDE
4,4'-DDD
4,4'-DDT
Endrin
Endosulfan sulfate
δ-BHC
Chlordane
Toxaphene
PCB-1242 (Aroclor 1242)
PCB-1254 (Aroclor 1254)
PCB-1221 (Aroclor 1221)
PCB-1232 (Aroclor 1232)
PCB-1248 (Aroclor 1248)
PCB-1260 (Aroclor 1260)
PCB-1016 (Aroclor 1016)

TABLE CA5. METALS AND OTHER COMPOUNDS

Metals, total	Others
Antimony	Asbestos
Arsenic	Cyanide
Beryllium	
Cadmium	
Chromium	
Copper	
Lead	
Mercury	
Nickel	
Selenium	
Silver	
Thallium	
Zinc	

APPENDIX CB

RAW DATA FROM THE AMES MUTAGENICITY TESTS AS DIRECTLY REPORTED BY MRC

Sample CTHF-3

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	A 1538
With Activation	Control		43, 50	51, 45	12, 18	19, 11	30, 25
	2-aminoanthracene	20 µg	847	1219	74	91	249
		10	49, 52	58, 45	14, 19	15, 16	16, 21
		50	49, 38	37, 35	18, 24	15, 17	26, 21
		100	48, 80	49, 44	23, 9	10, 10	16, 25
		500	43, 44	43, 45	10, 9	12, 14	26, 24
		1000	51, 43	47, 60	14, 9	6, 10	30, 19
Without Activation	Control		32, 35	190, 199	20, 10	12, 12	10, 10
	2-nitrofluorene	2.0 µg	11	—	—	—	322
	NaNO$_2$	9.0 mg	—	—	147	—	—
		10	22, 33	221, 241	23, 11	21, 18	11, 9
		50	35, 37	187, 256	12, 17	14, 11	3, 10
		100	32, 23	241, 264	8, 9	10, 9	6, 8
		500	16, 26	252, 295	4, 21	8, 15	5, 9
		1000	13, 10	221, 228	12,	14, 6	5, 6

Sample CTHF-3

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA 1538
With Activation	Control			158, 224			
	2-aminoanthracene	20 µg		1314			
		10		182, 137			
		50		163, 163			
		100		151, 164			
		500		163, 124			
		1000		168, 152			
Without Activation	Control						
	2-nitrofluorene	2.0 µg					
	$NaNO_2$	9.0 mg					

Sample CTHF-4

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	[illegible]
With Activation	Control		43, 50	51, 45	12, 8	19, 11	30, 25
	2-aminoanthracene	20 µg	3090	3591	477	364	2777
		10	40, 58	49, 43	17, 21	16, 19	39, 38
		50	41, 46	35, 23	23, 8	8, 10	39, 50
		100	36, 35	26, 42	10, 8	9, 12	26, 54
		500	53, 44	38, 35	9, 14	12, 12	31, 30
		1000	43, 35	33, 26	11, 7	14, 12	42, 23
Without Activation	Control		32, 35	190, 199	20, 10	12, 12	10, 10
	2-nitrofluorene	2.0 µg	11	—	—	—	322
	$NaNO_2$	9.0 mg	—	—	—	147	—
		10	46, 39	218, 212	17, 14	15, 16	8, 9
		50	44, 31	278, 294	15, 10	12, 17	9, 8
		100	42, 35	260, 181	18, 10	17, 10	2, 12
		500	33, 37	246, 269	10, 8	21, 11	10, 4
		1000	36, 32	180, 232	15, 9	14, 21	8, 11

Sample CTHF-4

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA 1538
With Activation	Control			158, 224			
	2-aminoanthracene	20 µg		1314			
		10		196, 172			
		50		185, 213			
		100		235, 196			
		500		227, 226			
		1000		168, 140			
Without Activation	Control						
	2-nitrofluorene	2.0 µg					
	$NaNO_2$	9.0 mg					

Sample CTHF - 5

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA 1538
With Activation	Control		65, 54	145, 198	26, 13	25, 14	28, 33
	2-aminoanthracene	20 µg	1712	1951	383	332	1911
		10	66, 48	221, 203	20, 19	17, 18	40, 33
		50	71, 65	237, 208	17, 19	16, 13	43, 39
		100	48, 57	210, 237	17, 15	16, 20	35, 40
		500	70, 48	220, 189	11, 15	17, 15	36, 35
		1000	58, 61	204, 190	11, 12	16, 13	30, 30
Without Activation	Control		8, 19	217, 114	24, 26	13, 16	14, 13
	2-nitrofluorene	2.0 µg	127	569	—	—	115
	$NaNO_2$	9.0 mg	—	—	353	—	—
		10	36, 31	252, 297	23, 20	13, 15	16, 15
		50	26, 35	292, 263	22, 23	19, 24	16, 19
		100	22, 25	236, 218	19, 24	12, 14	12, 20
		500	19, 12	157, 178	16, 19	15, 11	15, 16
		1000	18, 686	162, 96	14, 16	7, 12	19, 10

Sample CTHF - 7

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		65, 54	145, 198	12, 8	19, 11	30, 25
	2-aminoanthracene	20 µg	1712	1951	383	332	1911
		10	40, 52	192, 137	21, 16	12, 22	32, 18
		50	51, 65	177, 138	29, 13	16, 15	40, 40
		100	47, 56	163, 231	21, 9	10, 16	26, 37
		500	36, 34	136, 109	11, 17	10, 10	33, 35
		1000	27, 24	150, 130	13, 6	12, 10	33, 29
Without Activation	Control		8, 19	217, 114	24, 26	13, 16	14, 13
	2-nitrofluorene	2.0 µg	127	569	—	—	115
	$NaNO_2$	9.0 mg	—	—	353	—	—
		10	29, 26	213, 177	16, 12	12, 14	16, 11
		50	31, 21	182, 161	15, 20	10, 7	14, 13
		100	23, 29	227, 176	18, 15	13, 9	12, 14
		500	18, 15	112, 122	12, 18	13, 14	9, 8
		1000	19, 23	28, 94	8, 9	3, 13	10, 13

Sample CTHF - 8

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		64, 80	213, 205	16, 22	31, 32	38, 49
	2-aminoanthracene	20 µg	2240	2240	200	370	1700
		10	37, 47	167, 141	23, 27	26, 14	45, 40
		50	40, 44	187, 260	20, 25	21, 27	41, 37
		100	35, 38	184, 199	21, 12	15, 26	38, 35
		500	50, 51	127, 128	18, 16	14, 15	45, 43
		1000	39, 35	108, 49	14, 14	18, 13	46, 48
Without Activation	Control		66, 42	254, 261	33, 12	31, 18	27, 28
	2-nitrofluorene	2.0 µg	384	439	—	—	167
	$NaNO_2$	3.0 mg	—	—	383	—	—
		10	83, 54	266, 325	15, 19	17, 26	25, 22
		50	52, 50	336, 271	14, 14	20, 18	20, 20
		100	53, 55	255, 231	12, 12	30, 14	16, 12
		500	42, 52	258, 327	11, 14	18, 9	13, 19
		1000	60, 64	220, 184	12, 8	16, 16	9, 11

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Sample CTHF-9

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate				
			TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		64, 80	213, 205	16, 22	31, 32	38, 49
	2-aminoanthracene	20 µg	2240	2240	200	370	1700
		10	57, 74	244, 299	34, 17	28, 30	37, 51
		50	82, 68	303, 258	27, 17	21, 9	38, 42
		100	58, 56	216, 168	25, 22	25, 15	47, 38
		500	57, 56	272, 280	26, 16	22, 16	44, 37
		1000	16, 20	228, 214	17, 10	26, 13	21, 30
Without Activation	Control		66, 42	254, 261	33, 12	31, 18	27, 28
	2-nitrofluorene	2.0 µg	384	439	—	—	167
	$NaNO_2$	9.0 mg	—	—	383	—	—
		10	75, 73	381, 400	28, 27	16, 15	24, 29
		50	73, 53	352, 410	38, 18	17, 15	24, 18
		100	81, 59	353, 369	23, 31	25, 17	15, 19
		500	59, 48	333, 268	13, 24	22, 26	19, 5
		1000	47, 52	190, 169	12, 5	30, 22	35, 8

Sample CTHF - 10

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate				
			TA98	TA100	TA1535	TA1537	TA 1538
With Activation	Control		56, 76	287, 263	20, 32	10, 30	29, 29
	2-aminoanthracene	20 µg	3110	3818	443	464	1682
		10	61, 56	336, 380	26, 23	21, 25	3[illegible], 44
		50	71, 64	300, 321	16, 31	26, 20	45, 43
		100	60, 47	275, 204'	23, 27	30, 19	38, 35
		500	56, 67	278, 295	26, 17	20, 25	35, 32
		1000	53, 63	300, 291	20, 15	24, 30	38, 31
Without Activation	Control		40, 37	341, 391	15, 12	11, 16	27, 24
	2-nitrofluorene	2.0 µg	858	511	—	—	278
	$NaNO_2$	9.0 mg	—	—	—	—	—
		10	43, 63	471, 432	11, 21	8, 8	20, 22
		50	49, 51	378, 457	10, 11	16, 16	19, 14
		100	35, 51	383, 394	21, 22	8, 11	25, 23
		500	58, 51	398, 385	21, 22	8, 12	21, 21
		1000	60, 75	400, 385	23, 23	11, 14	22, 27

Sample CTHF - 11

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		71, 74	225, 204	17, 20	22, 16	35, 42
	2-aminoanthracene	20 µg	5097	4399	127	317	420
		10	55, 54	269, 225	29, 26	24, 23	56, 47
		50	63, 63	213, 229	30, 21	26, 29	42, 40
		100	64, 63	170, 219	18, 18	28, 22	64, 39
		500	67, 51	211, 212	17, 23	20, 17	42, 46
		1000	66, 54	195, 207	24, 20	18, 23	46, 38
Without Activation	Control		45, 81	338, 249	16, 24	11, 16	30, 25
	2-nitrofluorene	2.0 µg	425	460	—	—	152
	$NaNO_2$	9.0 mg	—	—	0	—	—
		10	81, 91	394, 329	12, 12	14, 14	30, 42
		50	110, 80	362, 317	12, 25	9, 14	36, 42
		100	79, 80	394, 318	19, 14	12, 7	32, 37
		500	106, 77	324, 332	19, 18	9, 4	36, 47
		1000	105, 108	306, 250	16, 15	9, 10	40, 56

Sample *CTHF - 11*

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	~~TA1537~~ TA1538
With Activation	Control						
	2-aminoanthracene	20 µg					
Without Activation	Control		8, 19				4,5
	2-nitrofluorene	2.0 µg	527				152
	NaNO$_2$	9.0 mg	—				—
		10	25, 22				15, 18
		50	31, 23				15, 19
		100	33, 29				17, 15
		500	26, 22				9, 15
		1000	29, 23				12, 16

Sample CTHF- 12

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		71, 74	225, 204	17, 20	22, 16	35, 42
	2-aminoanthracene	20 µg	5097	4399	127	317	420
		10	61, 70	240, 219	19, 18	16, 21	43, 33
		50	68, 54	232, 211	12, 12	23, 12	40, 42
		100	82, 65	256, 205	25, 10	24, 16	39, 40
		500	68, 60	256, 205	25, 10	24, 16	39, 40
		1000	55, 55	192, 202	14, 16	14, 15	40, 39
Without Activation	Control		45, 81	338, 249	16, 24	11, 16	7, 8
	2-nitrofluorene	2.0 µg	425	460	—	—	152
	$NaNO_2$	9.0 mg	—	—	0	—	—
		10	67, 83	389, 288	18, 17	7, 11	5, 3
		50	73, 71	332, 365	21, 22	15, 11	4, 2
		100	73, 81	320, 366	22, 22	15, 9	4, 1
		500	82, 81	298, 334	15, 14	15, 18	2, 3
		1000	92, 82	192, 297	15, 19	19, 11	2, 1

Sample CTHF-12

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control						
	2-aminoanthracene	20 µg					
Without Activation	Control		8, 19				4, 5
	2-nitrofluorene	2.0 µg	527				152
	NaNO$_2$	9.0 mg	—				—
		10	24, 30				12, 5
		50	60, 20				11, 10
		100	19, 31				8, 12
		500	41, 29				4, 13
		1000	21, 15				5, 8

Sample CTHF - 13

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		71, 74	225, 204	17, 20	22,16	35,42
	2-aminoanthracene	20 µg	5097	4399	127	317	420
		10	74,56	202, 306	16,15	17, 22	37,50
		50	63, 80	238, 219	21, 23	21, 22	36,40
		100	62, 74	269, 237	17, 24	25, 21	39,35
		500	69, 77	261, 228	12, 22	20, 19	22,21
		1000	71, 79	206, 227	19, 14	9, 10	24,41
Without Activation	Control		45, 81	338, 249	16, 24	11, 16	4, 5
	2-nitrofluorene	2.0 µg	425	460	—	—	152
	$NaNO_2$	9.0 mg	—	—	0	—	—
		10	70, 72	609, 468	21, 23	14, 10	29, 14
		50	82, 80	528, 424	20, 21	10, 14	28, 33
		100	96, 93	356, 311	22, 18	12, 5	22, 30
		500	81, 55	389, 253	12, 10	6, 5	5, 13
		1000	51, 71	165, 213	15, 19	10, 8	8, 5

Sample *CTHF -13*

Test Condition	Sample	Assay Amount (µl/plate)	TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control						
	2-aminoanthracene	20 µg					
Without Activation	Control		8, 19				7, 8
	2-nitrofluorene	2.0 µg	527				303
	NaNO$_2$	3.0 mg	—				—
		10	26, 24				2, 4
		50	28, 23				2, 3
		100	25, 36				6, 0
		500	26, 30				0, 1
		1000	30, 29				7, 4

Reproduced from best available copy.

Sample CTHF - 14

Test Condition	Sample	Assay Amount (µl/plate)	TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		48, 31	59, 60	9, 9	11, 12	25, 25
	2-aminoanthracene	20 µg	3844	3557	330	262	3254
		10	36, 33	73, 95	29, 12	13, 15	29, 21
		50	28, 32	64, 79	10, 9	18, 10	24, 26
		100	40, 53	65, 57	11, 11	18, 11	27, 25
		500	38, 42	81, 72	19, 12	11, 9	25, 30
		1000	44, 20	72, 69	8, 12	5, 9	18, 21
Without Activation	Control		12, 14	190, 199	10, 21	10, 11	4, 7
	2-nitrofluorene	2.0 µg	409	—	—	—	264
	$NaNO_2$	9.0 mg	—	—	182	—	—
		10	29, 30	201, 249	16, 15	21, 8	13, 10
		50	16, 25	253, 291	11, 9	9, 11	12, 13
		100	27, 26	254, 268	8, 11	18, 5	10, 11
		500	24, 23	250, 277	8, 18	12, 10	11, 8
		1000	5, 7	238, 250	3, 3	8, 9	8, 9

Sample *CTHF-14*

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control			158, 224			
	2-aminoanthracene	20 µg		1314			
		10		288, 197			
		50		234, 203			
		100		222, 243'			
		500		207, 246			
		1000		136, 169			
Without Activation	Control						
	2-nitrofluorene	2.0 µg					
	$NaNO_2$	9.0 mg					

Sample *CTHF- 15*

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate				
			TA98	TA100	TA1535	TA1537	TA 1538
With Activation	Control		48, 31	158, 224	9, 9	11, 12	26, 25
	2-aminoanthracene	20 µg	3844	1314	330	262	3254
		10	44, 40	267, 242	17, 23	12, 13	15, 25
		50	39, 36	177, 168	11, 17	5, 15	25, 25
		100	58, 38	128, 171	5, 10	18, 11	24, 22
		500	45, 49	189, 177	7, 11	20, 18	18, 21
		1000	28, 29	143, 135	14, 9	10, 16	30, 15
Without Activation	Control		7, 6	190, 199	10, 21	10, 11	4, 7
	2-nitrofluorene	2.0 µg	368	—	—	—	264
	NaNO$_2$	9.0 mg	—	—	182	—	—
		10	8, 7	—	15, 10	16, 9	10, 11
		50	26, 11	278, 290	15, 15	11, 7	12, 10
		100	16, 20	206, 228	23, 8	17, 11	10, 10
		500	8, 16	255, 248	7, 15	22, 15	11, 15
		1000	7, 11	219, 238	4, 13	5, 12	9, 10

Sample CTHF - 15

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate				
			TA98	TA100	TA1535	TA1537	TA 1538
With Activation	Control						
	2-aminoanthracene	20 µg					
Without Activation	Control						7, 8
	2-nitrofluorene	2.0 µg					303
	$NaNO_2$	9.0 mg					—
		10					10, 7
		50					9, 5
		100					6, 8
		500					9, 2
		1000					5, 4

Sample CTHF-16

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate				
			TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control		59, 53	56, 56	8, 11	9, 15	33, 40
	2-aminoanthracene	20 µg	2843	1961	425	85	436
		10	39, 45	59, 30	12, 13	19, 36	15, 18
		50	54, 45	35, 43	16, 19	25, 43	15, 16
		100	32, 59	52, 43	15, 16	23, 25	16, 32
		500	45, 57	45, 33	15, 14	20, 20	47, 29
		1000	37, 18	47, 26	8, 7	22, 12	25, 24
Without Activation	Control		8, 19	190, 199	18, 12	11, 16	15, 10
	2-nitrofluorene	2.0 µg	527	—	—	—	711
	$NaNO_2$	9.0 mg	—	—	68	—	—
		10	28, 18	256, 324	14, 10	16, 10	8, 7
		50	28, 30	134, 310	19, 20	15, 11	10, 12
		100	25, 28	221, 308	16, 16	8, 11	10, 5
		500	22, 32	248, 233	14, 10	9, 9	16, 8
		1000	10, 38	155, 142	18, 9	3, 3	9, 5

Sample CTHF-16

Test Condition	Sample	Assay Amount (µl/plate)	Revertants Per Plate				
			TA98	TA100	TA1535	TA1537	TA1538
With Activation	Control			158, 224			
	2-aminoanthracene	20 µg		1314			
		10		137, 149			
		50		151, 187			
		100		183, 143'			
		500		154, 149			
		1000		159, 91			
Without Activation	Control						
	2-nitrofluorene	2.0 µg					
	$NaNO_2$	9.0 mg					

APPENDIX CC

RAW DATA FOR THE CHO CYTOTOXICITY TESTS AS DIRECTLY REPORTED BY MRC

CYTOTOXICITY DATA FOR CADMIUM CHLORIDE STANDARD 6/5/78
CELL LINE: CHO PAGE REF: 1207185

CONTROL BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
430	440	8
440		
450		
445		
430		
445		

CONCENTRATION (MG/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.001	0 0 0	0	0	0
.0005	0 0 0	0	0	0
.0004	0 0 0	0	0	0
.0003	0 0 0	0	0	0
.0002	0 0 0	0	0	0
.0001	90 15 15	40	43	9

BASIC

MONSANTO COMPANY № 1214106

BJECT [illegible]

OB NO.	PREPARED BY (SIGNATURE)	DATE
	[illegible]	[illegible]

OTOXICITY DATA FOR CADMIUN CHLORIDE
L LINE: CHO

6/22/78
PAGE REF: 1214103

CONTROL CKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
421	435	60
369		
374		
524		
449		
473		

NCENTRATION (MG/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.001	0 0 0	0	0	0
.0005	0 0 0	0	0	0
.0004	0 0 0	0	0	0
.0003	0 0 0	0	0	0
0002	2 22 6	10	11	2
.0001	78 409 449	312	204	100

READ AND UNDERSTOOD BY	DATE

```
CYTOTOXICITY DATA FOR CADMIUM CHLORIDE STANDARD         7/6/78
CELL LINE: CHO                                          PAGE REF: 1214115

   CONTROL
(BACKGROUND)                      MEAN                         STANDARD
   VALUES                         VALUE                        DEVIATION
------------                      -----                        ---------
     625                           617                            8
     611
     614
     607
     627
     615

CONCENTRATION        REPLICATE      MEAN       STANDARD       PERCENT
   (MG/ML)             VALUES       VALUE      DEVIATION      SURVIVAL
-------------        ---------      ------     ---------      --------

  .001                  0            0            0              0
                        0
                        0

  .0005                 0            0            0              0
                        0
                        0

  .0004                 7            8            1              1
                        7
                        9

  .0003                25           21            7              3
                       13
                       25

  .0002               573          575           66            100
                      642
                      511

  .0001               647          638           20            100
                      615
                      653

BASIC
>
```

CYTOTOXICITY DATA FOR CADMIUM CHLORIDE STANDARD
CELL LINE: CHO

7/11/78
PAGE REF: 1214126

CONTROL BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
557	536	16
547		
518		
520		
528		
546		

CONCENTRATION (MG/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.001	0 0 0	0	0	0
.0005	0 0 0	0	0	0
.0004	0 0 0	0	0	0
.0003	0 0 0	0	0	0
.0002	57 32 12	34	23	6
.0001	520 521 508	516	7	100

BASIC

CYTOTOXICITY DATA FOR CTHF-3
CELL LINE: CHO

7/6/78
PAGE REF: 1212115

CONTROL BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
625	617	8
611		
614		
607		
627		
615		

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	687 707 725	706	19	100
150	723 733 685	714	25	100
100	702 696 707	702	6	100
50	738 684 655	692	42	100
10	643 691 652	662	26	100
2	586 577 584	582	5	94
.2	609 569 608	595	23	100

BASIC

C1

```
CYTOTOXICITY DATA FOR CTFH-4                            7/6/78
CELL LINE: CHO                                          PAGE REF: 1214115

  CONTROL
(BACKGROUND)                       MEAN                            STANDARD
  VALUES                           VALUE                           DEVIATION
------------                       -----                           ---------
   624                              617                               8
   612
   614
   607
   627
   615

CONCENTRATION        REPLICATE      MEAN          STANDARD        PERCENT
   (UL/ML)            VALUES        VALUE         DEVIATION       SURVIVAL
-------------        ---------      ------        ---------       --------

  200                   711          718            14               100
                        734
                        709

  150                   685          683            13               100
                        670
                        695

  100                   674          658            14               100
                        652
                        647

  50                    625          638            11               100
                        644
                        645

  10                    647          602            49               100
                        608
                        550

  2                     582          598            16               100
                        597
                        614

 .2                     613          608            8                100
                        611
                        599

BASIC
```

CYTOTOXICITY DATA FOR CTHF-5
CELL LINE: CHO

7/6/78
PAGE REF: 1214115

CONTROL BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
625	617	8
611		
614		
607		
627		
615		

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	616	649	29	100
	665			
	667			
150	664	650	13	100
	638			
	647			
100	626	655	26	100
	678			
	660			
50	635	627	12	100
	613			
	634			
10	628	611	21	100
	617			
	587			
2	608	613	15	100
	602			
	630			
.2	614	602	12	100
	602			
	590			

BASIC

MONSANTO COMPANY № 1214107

SUBJECT CHO - Clemson CTHF-6		
JOB NO.	PREPARED BY (SIGNATURE)	DATE 6/22/78

YTOTOXICITY DATA FOR CTHF-6
ELL LINE: CHO

6/22/78
PAGE REF: 1214103

CONTROL BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
421	435	60
369		
374		
524		
449		
473		

ONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	6 4 4	5	1	1
150	20 5 10	12	8	3
100	122 166 170	153	27	35
50	396 424 378	399	23	100
10	485 469 453	469	16	100
2	466 387 256	370	106	100
.2	245 363 212	273	79	63

READ AND UNDERSTOOD BY	DATE

MONSANTO COMPANY № 1214149

SUBJECT CHO- Clemson Samples		
JOB NO. 13.022 760.57 105.0519	PREPARED BY (SIGNATURE)	DATE 7/25/78

CYTOTOXICITY DATA FOR CTHF-6
CELL LINE: CHO

7/25/78
PAGE REF: 1214143

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
497	478	15
480		
484		
450		
480		
478		

CENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	0 16 17	11	10	2
150	88 90 96	91	4	19
100	220 221	221	1	46
50	419 431	425	8	89
10	454 300 220	325	119	68
2	467 436 450	451	16	100
.2	480 463 473	472	9	100

READ AND UNDERSTOOD BY	DATE

YTOTOXICITY DATA FOR CTHF-7
ELL LINE: CHO

7/6/78
PAGE REF: 1214116

CONTROL BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
625	617	8
611		
614		
607		
627		
615		

ONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	706	694	16	100
	700			
	675			
150	695	650	45	100
	650			
	605			
100	655	668	23	100
	695			
	654			
50	633	632	4	100
	628			
	635			
10	615	623	12	100
	637			
	618			
5	623	607	24	100
	619			
	580			
[illegible]	595	605	11	100
	602			
	617			

6511

```
CYTOTOXICITY DATA FOR CTHF-8                                  7/6/78
CELL LINE: CHO                                                PAGE REF: 1214116


  CONTROL
(BACKGROUND)                           MEAN                                STANDARD
  VALUES                               VALUE                               DEVIATION
------------                           -----                               ---------
    625                                 617                                    8
    611
    614
    607
    627
    615


CONCENTRATION          REPLICATE        MEAN           STANDARD           PERCENT
   (UL/ML)              VALUES          VALUE          DEVIATION          SURVIVAL
-------------          ---------        ------         ---------          --------

   200                    677            658              34                 100
                          678
                          618

   150                    604            587              15                  95
                          581
                          575

   100                    631            632              18                 100
                          650
                          615

   50                     640            620              20                 100
                          619
                          601

   10                     594            601               6                  97
                          604
                          605

   2                      631            620              13                 100
                          606
                          624

   .2                     606            622              14                 100
                          626
                          633

BASIC
```

C12

```
CYTOTOXICITY DATA FOR CTHF-9                                  7/6/78
CELL LINE: CHO                                                PAGE REF: 1214116

   CONTROL
 (BACKGROUND)                          MEAN                        STANDARD
   VALUES                              VALUE                       DEVIATION
------------                           -----                       ---------
     625                                617                           8
     611
     614
     607
     627
     615

CONCENTRATION         REPLICATE          MEAN          STANDARD         PERCENT
   (UL/ML)              VALUES           VALUE         DEVIATION        SURVIVAL
-------------         ---------          ------        ---------        --------

  200                    680              706             30              100
                         739
                         700

  150                    730              726             12              100
                         735
                         712

  100                    716              696             19              100
                         678
                         694

  50                     714              695             19              100
                         695
                         677

  10                     616              605             13              100
                         609
                         590

  2                      591              609             16              100
                         620
                         617

  .2                     609              625             15              100
                         638
                         627

 BASIC
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MONSANTO COMPANY № 1214108

SUBJECT
CHO - Clemson CTHF-10

JOB NO.	PREPARED BY (SIGNATURE)	DATE
	DM	6/22/78

CYTOTOXICITY DATA FOR CTHF-10
CELL LINE: CHO

6/22/78
PAGE REF: 1214103

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
421 369 374 524 449 473	435	60

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	291 234 159	228	66	52
150	495 192 468	385	168	100
100	492 484 473	483	10	100
50	486 536 507	510	25	100
10	491 497 456	481	22	100
2	94 110 96	100	9	23
.2	450 465 433	449	16	100

READ AND UNDERSTOOD BY	DATE
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CYTOTOXICITY DATA FOR CTHF-10                                     7/11/78
CELL LINE: CHO                                                    PAGE REF: 1214126

   CONTROL
 (BACKGROUND)                       MEAN                               STANDARD
   VALUES                           VALUE                              DEVIATION
 ------------                       -----                              ---------
     557                             536                                  16
     547
     518
     520
     528
     546


CONCENTRATION        REPLICATE       MEAN           STANDARD          PERCENT
   (UL/ML)            VALUES         VALUE          DEVIATION         SURVIVAL
-------------        ---------       ------         ---------         --------

  200                   596           588              20                100
                        603
                        565

  150                   558           552               8                100
                        543
                        554

  100                   550           538              11                100
                        532
                        531

  50                    518           525              16                100
                        544
                        514

  10                    500           511              11                100
                        521
                        511

  2                     524           519               5                100
                        518
                        515

  .2                    532           530               2                100
                        528
                        530

BASIC
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CYTOTOXICITY DATA FOR CTHF-11                                  7/11/78
CELL LINE: CHO                                                 PAGE REF: 1214126


     CONTROL
  (BACKGROUND)                     MEAN                               STANDARD
     VALUES                        VALUE                              DEVIATION
  ------------                     -----                              ---------
       557                          536                                  16
       547
       518
       520
       528
       546


CONCENTRATION        REPLICATE      MEAN          STANDARD         PERCENT
   (UL/ML)            VALUES        VALUE         DEVIATION        SURVIVAL
-------------        ---------      ------        ---------        --------

   200                  566          564             18              100
                        580
                        545

   150                  549          531             16              100
                        526
                        518

   100                  476          489             18               91
                        482
                        510

   50                   535          515             23              100
                        490
                        520

   10                   524          548             23              100
                        570
                        550

   2                    514          510             19              100
                        527
                        490

   .2                   515          522             7               100
                        523
                        528

BASIC
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CYTOTOXICITY DATA FOR CTHF-12                              7/11/78
CELL LINE: CHO                                             PAGE REF: 1214126


   CONTROL
 (BACKGROUND)                      MEAN                               STANDARD
   VALUES                          VALUE                              DEVIATION
------------                       -----                              ---------
     557                            536                                 16
     547
     518
     520
     528
     546


CONCENTRATION        REPLICATE        MEAN          STANDARD         PERCENT
   (UL/ML)             VALUES         VALUE         DEVIATION        SURVIVAL
-------------        ---------        ------        ---------        --------

  200                   530            531             14               100
                        518
                        545

  150                   550            542             23               100
                        560
                        516

  100                   542            546             24               100
                        572
                        524

  50                    513            531             20               100
                        528
                        552

  10                    456            480             32               90
                        517
                        468

  2                     483            503             28               100
                        490
                        535

  .2                    520            511             23               100
                        485
                        528

BASIC
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CYTOTOXICITY DATA FOR CTHF-13                        6-12-78
CELL LINE: CHO-K1                                    PAGE REF: 1207186

   CONTROL
(BACKGROUND)                     MEAN                        STANDARD
   VALUES                        VALUE                       DEVIATION
------------                     -----                       ---------
    430                           440                          8
    445
    430
    440
    450
    445


CONCENTRATION         REPLICATE        MEAN        STANDARD         PERCENT
   (UL/ML)              VALUES         VALUE       DEVIATION        SURVIVAL
-------------         ---------        ------      ---------        --------

  200                    0              0            0                0
                         0
                         0

  150                    30             42           25               9
                         70
                         25

  100                    260            203          53               46
                         195
                         155

  50                     370            445          106              100
                         520

  2                      470            470          0                100

  .2                     450            450          0                100

BASIC
>
```

CYTOTOXICITY DATA FOR CTHF-13
CELL LINE: CHO

7/11/78
PAGE REF: 1214|26

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
557 547 518 520 528 546	536	16

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	94 170 222	162	64	30
150	440 451 383	425	37	79
100	540 521 541	534	11	100
50	570 574 531	558	24	100
10	528 530 525	528	3	100
2	514 515 500	510	8	95
.2	507 485 514	502	15	94

BASIC

C14

MONSANTO COMPANY № 1214112

SUBJECT
CHO Clemson - CTHF-14

JOB NO.
105-0519

PREPARED BY (SIGNATURE)
DM Levane

DATE
6/22/78

CYTOTOXICITY DATA FOR CTHF-14
CELL LINE: CHO

6/22/78
PAGE REF: 1214104

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
421	435	60
369		
374		
524		
449		
473		

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	515 483 505	501	16	100
150	513 482 539	511	29	100
100	325 181 430	312	125	100
50	492 470 465	476	14	100
10	491 515 457	488	29	100
2	384 494 385	421	63	100
.2	484 473 440	466	23	100

READ AND UNDERSTOOD BY

DATE

CYTOTOXICITY DATA FOR CTHF-15 7/11/78
CELL LINE: CHO PAGE REF: 1214126

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
557 547 518 520 546 528	536	16

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	556 547 530	544	13	100
150	557 570 528	552	22	100
100	559 541 517	539	21	100
50	473 482 518	491	24	92
10	491 503 492	495	7	92
2	512 478 524	505	24	100
.2	539 526 540	535	8	100

ASIC

MONSANTO COMPANY № 1214114

SUBJECT CHO - [illegible] CTHF - 16		
JOB NO. 106-0519	PREPARED BY (SIGNATURE) D McLean	DATE 6/22/78

:YTOTOXICITY DATA FOR CTHF-16 6/22/78
:ELL LINE: ABORT PAGE REF: 1214105

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
421	435	60
369		
374		
524		
449		
473		

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
200	354 254 320	309	51	71
150	472 483 471	475	7	100
100	462 373 379	405	50	100
50	395 386 283	355	62	100
10	420 452 450	441	18	100
2	460 272 403	378	96	100
.2	423 336 437	399	55	100

READ AND UNDERSTOOD BY	DATE

APPENDIX CD

CHARACTERISTICS OF THE 14 WASTEWATER SAMPLES AND RECONSTITUTED WATER AS DIRECTLY REPORTED BY EG&G BIONOMICS

Table CD1 -- Characteristics of CTHF-3 effluent measured on 16 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 15 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	dark red-purple liquid
pH:	10.1
DO (mg/ℓ):	10.2
Temperature (°C):	18
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	540
Reconstituted Water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	45
Total alkalinity as $CaCO_3$ (mg/ℓ):	31
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the four, 5-gallon containers.

Table CD1-- Characteristics of CTHF-4 effluent measured on 23 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 21 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	clear liquid
pH:	8.5
DO (mg/ℓ):	10.5
Temperature (°C):	23
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	20
Reconstituted water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	46
Total alkalinity as $CaCO_3$ (mg/ℓ):	32
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the four, 5-gallon containers.

Table CD1-- Characteristics of CTHF-5 effluent measured on 23 June 1978, received from Clemson University, Clemson, South Carolina on 21 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	clear liquid
pH:	7.9
DO (mg/ℓ):	11
Temperature (°C):	23
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	11
Reconstituted water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	46
Total alkalinity as $CaCO_3$ (mg/ℓ):	32
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the four, 5-gallon containers.

Table CD1-- Characteristics of CTHF-6 effluent measured on 19 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 15 June 1978. The reconstituted water is also characterized.

Parameter	Effluent
Physical description:	cloudy, orange-brown liquid
pH:	9.9
DO (mg/ℓ):	10.8
Temperature (°C):	13
Salinity (o/oo):	5[a]
Specific conductance (μmhos/cm):	418
Reconstituted Water	
pH:	7.5
Total hardness as $CaCO_3$ (mg/ℓ):	44
Total alkalinity as $CaCO_3$ (mg/ℓ):	31
Specific conductance (μmhos/cm):	147

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[a] Salinity was measured with an American Optical refractometer.

Table CD1-- Characteristics of CTHF-7 effluent measured on 19 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 15 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	clear liquid
pH:	10.7
DO (mg/ℓ):	10.3
Temperature (°C):	11
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	1,003
Reconstituted Water	
pH:	7.6[b]
Total hardness as $CaCO_3$ (mg/ℓ):	45
Total alkalinity as $CaCO_3$ (mg/ℓ):	31
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the four, 5-gallon containers.

[b] Values of water used for test series conducted between 20-24 June 1978.

Table CD1-- Characteristics of CTHF-8 effluent measured on 23 June 1978, received from Clemson University, Clemson, South Carolina on 21 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	clear liquid
pH:	9.2
DO (mg/ℓ):	8.9
Temperature (°C):	22
Salinity (o/oo):	0
Specific conductance (µmhos/cm):	240
Reconstituted Water	
pH:	7.6(7.7)[b]
Total hardness as $CaCO_3$ (mg/ℓ):	46(43)[b]
Total alkalinity as $CaCO_3$ (mg/ℓ):	32(33)[b]
Specific conductance (µmhos/cm):	145(163)[b]

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[a] Parameters measured before testing, after combining all effluent containers.

[b] Parameters are for 100% effluent solution set up on 30 June 1978.

Table CD1-- Characteristics of CTHF-9 effluent measured on 23 June 1978, received from Clemson University, Clemson, South Carolina on 21 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	light yellow colored liquid
pH:	9.3
DO (mg/ℓ):	9.7
Temperature (°C):	22
Salinity (o/oo):	0.75
Specific conductance (μmhos/cm):	1,950
Reconstituted Water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	46
Total alkalinity as $CaCO_3$ (mg/ℓ):	32
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the five, 1-gallon containers.

Table CD1-- Characteristics of CTHF-10 effluent measured on 9 June 1978, received from Clemson University, Clemson, South Carolina on 9 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	a light green-brown, slightly cloudy, liquid
pH:	6.8
DO (mg/ℓ):	6.9
Temperature (°C):	16
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	319
Reconstituted water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	42
Total alkalinity as $CaCO_3$ (mg/ℓ):	30
Specific conductance (μmhos/cm):	145

[a] Parameters measured before testing, after combining the four, five gallon containers.

Table CD1 -- Characteristics of CTHF-11 effluent measured on 9 June 1978, received from Clemson University, Clemson, South Carolina on 9 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	clear liquid
pH:	6.6
DO (mg/ℓ):	9.3
Temperature (^{o}C):	16
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	44
Reconstituted water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	42
Total alkalinity as CaCO (mg/ℓ):	30
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the four, 5-gallon containers.

Table CD1 -- Characteristics of CTHF-12 effluent measured on 9 June 1978, received from Clemson University, Clemson, South Carolina on 9 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	Clear liquid
pH:	6.5
DO (mg/ℓ):	9.1
Temperature (°C):	16
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	22
Reconstituted water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	30
Total alkalinity as $CaCO_3$ (mg/ℓ):	42
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the 4, 5-gallon containers.

Table CD1-- Characteristics of CTHF-13 effluent measured on 9 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 9 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	a dark brown, slightly cloudy liquid
pH:	7.7
DO (mg/ℓ):	8.4
Temperature (°C):	16
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	1,520
Reconstituted Water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	42
Total alkalinity as $CaCO_3$ (mg/ℓ):	30
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the two, 5-gallon containers.

TableCD1 -- Characteristics of CTHF-14 effluent measured on 16 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 15 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	pale yellow liquid
pH:	7.1
DO (mg/ℓ):	4.6
Temperature (°C):	18
Salinity (o/oo):	0
Specific conductance (μmhos/cm):	680
Reconstituted Water	
pH:	7.3
Total hardness as $CaCO_3$ (mg/ℓ):	43
Total alkalinity as $CaCO_3$ (mg/ℓ):	30
Specific conductance (μmhos/cm):	164

[a] Parameters measured before testing, after combining the four, 5-gallon containers.

Table D1-- Characteristics of CTHF-15 effluent measured on 16 June 1978, received from Clemson University, Clemson, South Carolina on 15 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	a pale orange colored liquid
pH:	8.3
DO (mg/ℓ):	9.7
Temperature (°C):	19
Salintiy (o/oo):	0
Specific conductance (μmhos/cm):	95
Reconstituted Water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	45
Total alkalinity as $CaCO_3$ (mg/ℓ):	31
Specific conductance (μmhos/cm):	145

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[a] Parameters measured before testing, after combining the four, 5-gallon containers.

Table CD1-- Characteristics of CTHF-16 effluent measured on 19 June 1978, received from the Monsanto Research Corporation, Dayton, Ohio on 15 June 1978. The reconstituted water is also characterized.

Parameter	Effluent[a]
Physical description:	a dark red liquid
pH:	8.5
DO (mg/ℓ):	9.8
Temperature (°C):	11
Salinity (o/oo):	0
Specific conductance (µmhos/cm):	2,255
Reconstituted Water	
pH:	7.6
Total hardness as $CaCO_3$ (mg/ℓ):	45
Total alkalinity as $CaCO_3$ (mg/ℓ):	31
Specific conductance (µmhos/cm):	145

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[a] Parameters measured before testing, after combining the two, 5-gallon containers.

APPENDIX CE

CHARACTERISTICS OF THE WASTEWATER SAMPLES AS A FUNCTION OF TIME AND MORTALITY DOSE RESPONSE DATA AS DIRECTLY REPORTED BY EG&G BIONOMICS FOR FATHEAD MINNOR ACUTE TOXICITY TESTS

Table C-2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-3 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	53	9.9	9.7	9.2	8.7	8.2
	5.3	8.7	7.5	7.0	7.0	7.0
	0.24	7.4	7.3	7.1	7.0	7.1
	control	7.2	7.1	7.0	7.0	7.1
DO (mg/ℓ)	53	9.4(>100)[a]	9.0(100)	0.2(2.3)	0.4(45)	0.3(3.4)
	5.3	9.0(>100)	6.1(69)	2.1(24)	1.9(22)	2.0(23)
	0.24	8.8(100)	7.8(89)	5.2(59)	5.3(60)	5.5(62)
	control	8.7(99)	7.5(85)	4.8(55)	5.2(59)	5.4(61)
specific conductance (µmhos/cm)	53	378				
	5.3	189				
	0.24	158				
	control	155				
total hardness as $CaCO_3$ (mg/ℓ)	53	28				
	5.3	44				
	0.24	42				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	53	92				
	5.3	37				
	0.24	32				
	control	30				

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[a] % of saturation at 22°C.

Table CE4-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-3 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
53	100	100	100	100
24	90[a]	100	100	100
11	0[b]	0[b]	0[b]	0[c]
5.3	0[b]	0[b]	0[b]	0[b]
2.4	0	0	0	0[b]
1.1	0	0	0	0[b]
0.53	0	0	0	0
0.24	0	0	0	0
control	0	0	0	0

[a] One fish showed a complete loss of equilibrium.

[b] Fish were lethargic.

[c] Fish were at the surface.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-4 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	36	7.6	7.0	7.5	6.9	7.0
	7.8	7.6	7.0	7.5	7.0	7.0
	1.7	7.5	7.0	7.6	7.1	7.0
	control	7.5	6.9	7.6	7.0	7.1
DO (mg/ℓ)	36	9.3(>100)[a]	6.9(78)	5.3(60)	4.5(51)	5.2(59)
	7.8	9.1(>100)	6.9(78)	4.3(49)	3.7(42)	4.2(48)
	1.7	9.0(>100)	6.8(77)	4.5(51)	3.6(41)	4.0(45)
	control	8.9(>100)	6.7(76)	4.5(51)	3.6(41)	3.5(40)
specific conductance (μmhos/cm)	36	105				
	7.8	141				
	1.7	147				
	control	149				
total hardness as $CaCO_3$ (mg/ℓ)	36	28				
	7.8	42				
	1.7	44				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	36	21				
	7.8	29				
	1.7	29				
	control	30				

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[a] % of saturation at 22°C.

Table CE4-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-4 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
36	100	100	100	100
22	10[a]	30[a,b,c]	40[b,c]	40[b,c]
13	0[a,b]	0[b]	0[b]	0[b]
7.8	0	0	0	0[b]
4.6	0	0	0	0
2.8	0	0	0	0
1.7	0	0	0	0
control	0	0	0	0

[a] Some fish displayed a loss of equilibrium.

[b] Fish were lethargic.

[c] Fish displayed a dark coloration.

TableCE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-5 effluent and fathead minnow (*Pimephales promelas*).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	100	7.0	6.9	6.1	6.6	6.5
	36	7.5	7.2	6.6	6.5	6.8
	7.7	7.6	7.2	6.7	6.7	6.8
	control	7.3	7.2	6.8	6.5	6.8
DO (mg/ℓ)	100	11(>100)[a]	7.6(86)	5.6(64)	2.3(26)	1.6(18)
	36	9.8(>100)	6.8(77)	3.8(43)	3.4(39)	4.1(47)
	7.7	9.3(>100)	6.6(75)	3.9(44)	3.7(42)	3.6(41)
	control	8.9(>100)	5.1(58)	3.7(42)	3.5(40)	3.5(40)
specific conductance (μmhos/cm)	100	23				
	36	110				
	7.7	140				
	control	140				
total hardness as $CaCO_3$ (mg/ℓ)	100	1				
	36	28				
	7.7	40				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	100	6				
	36	22				
	7.7	28				
	control	29				

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[a] % of saturation at 22°C.

Table CE3-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-5 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
100	0[a]	30	30	30
60	0	0	0	0
36	0	0	0	0
22	0	0	0	0
13	0	0	0	0
7.7	0	0	0	0
control	0	0	0	0

[a] One fish displayed a complete loss of equilibrium.

Table CE2- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-6 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	5.3	9.4	9.0	8.3	7.9	7.4
	0.53	8.4	7.4	7.2	7.2	7.1
	0.053	7.8	7.3	7.1	7.2	7.1
	control	7.7	7.3	7.2	7.2	7.1
DO (mg/ℓ)	5.3	9.1(>100)	2.7(31)	0.1(1.1)	0.2(2.3)	0.4(4.5)
	0.53	9.1(>100)	6.2(70)	4.7(53)	4.9(56)	4.8(55)
	0.053	9.0(>100)	7.2(82)	6.0(68)	6.0(68)	5.5(62)
	control	9.1(>100)	7.3(83)	6.1(69)	6.0(68)	5.6(64)
specific conductance (μmhos/cm)	5.3	369				
	0.53	176				
	0.053	153				
	control	150				
total hardness as $CaCO_3$ (mg/ℓ)	5.3	50				
	0.53	44				
	0.053	44				
	control	46				
total alkalinity as $CaCO_3$ (mg/ℓ)	5.3	89				
	0.53	37				
	0.053	34				
	control	32				

[a] % of saturation at 22°C.

Table CE4-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-6 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration[a] (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
5.3	100	100	100	100
2.4	30[b,c]	100	100	100
1.1	0	0	0	0[d]
0.53	0[b]	0[b,d]	0[b]	0
0.24	0	0	0	0
0.11	0	0	0	0
0.053	0	0	0	0
control	0	0	0	0

[a] All effluent test solutions were cloudy in proportion to the concentration for the duration of the test.

[b] One fish displayed a dark coloration.

[c] Some of the fish showed a complete loss of equilibrium.

[d] Fish were lethargic.

TableCE2-- The pH, DO, specific conductance, total hardness and alklainity measurements made during a 96-hour toxicity determination with CTHF-7 effluent and fathead minnow (*Pimephales promelas*).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	100	10.6	10.1	9.7	9.5	9.3
	46	10.2	9.8	9.2	8.9	8.5
	7.7[a]	9.1	8.3	7.4	7.2	7.2
	control	7.6	7.2	7.1	7.1	7.0
	control[a]	7.0	7.0	7.0	7.1	7.3
DO (mg/ℓ)	100	11.7(>100)[b]	10.3(>100)	1.8(20)	0.1(1.1)	0.1(1.1)
	46	10.3(>100)	9.2(>100)	0.3(34)	0.2(2.3)	0.3(3.4)
	7.7[a]	9.1(>100)	5.0(57)	1.2(14)	2.1(24)	3.2(36)
	control	9.1(>100)	6.4(73)	4.0(45)	3.9(44)	4.0(45)
	control[a]	8.6(98)	5.1(58)	4.3(49)	4.3(49)	4.3(49)
specific conductance (µmhos/cm)	100	890				
	46	461				
	7.7[a]	196				
	control	156				
	control[a]	131				
total hardness as $CaCO_3$ (mg/ℓ)	100	14				
	46	30				
	7.7[a]	42				
	control	46				
	control[a]	38				
total alkalinity as $CaCO_3$ (mg/ℓ)	100	242				
	46	128				
	7.7[a]	48				
	control	32				
	control[a]	28				

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[a] These test solutions were conducted between 20 and 24 June 1978.

[b] % of saturation at 22°C.

Table CE4 -- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-7 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration[a] (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
100	100	100	100	100
68	100	100	100	100
46	100	100	100	100
32	100	100	100	100
22	70[b]	100	100	100
7.8[c]	0	10	10	10
control	0	0	0	0
control[c]	0	10	10	10[d]

[a] All effluent test solutions were cloudy in proportion to the concentration for the duration of the test.

[b] Some fish showed a complete loss of equilibrium.

[c] There test solutions were conducted between 20 and 24 June 1978.

[d] Fish were lethargic.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-8 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	100	9.2	9.2	8.9	–[a]	–[a]
	13	8.8	7.5	6.7	6.7	6.9
	1.9	7.9	7.2	6.7	6.7	6.8
	control[b]	7.1	7.2	7.0	–[a]	–[a]
	control	7.6	7.1	6.7	6.6	6.8
DO (mg/ℓ)	100	12(>100)[c]	8.0(91)	5.3(60)	–[a]	–[a]
	13	9.3(>100)	5.8(66)	2.7(31)	2.7(31)	2.7(31)
	1.9	9.2(>100)	6.6(75)	4.2(48)	4.1(47)	3.7(42)
	control[b]	8.8(100)	7.9(90)	5.7(65)	–[a]	–[a]
	control	9.1(>100)	7.0(80)	4.6(52)	4.6(52)	4.5(51)
specific conductance (μmhos/cm)	100	237				
	13	160				
	1.9	150				
	control[b]	157				
	control	150				
total hardness as $CaCO_3$ (mg/ℓ)	100	1				
	13	38				
	1.9	42				
	control[b]	41				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	100	109				
	13	39				
	1.9	33				
	control[b]	31				
	control	29				

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[a] Measurements not made due to technician error.

[b] This control set on 30 June with 100% effluent solution.

[c] % of saturation at 22°C.

Table CE3-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-8 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
100	0	0[a]	0	0
88	0[b]	0	10[c]	10
46	0	0	0	0
24	0	0	0	0
13	0	0	0	0
6.8	0	0	0	0
3.6	0	0	0	0
1.9	0	0	0	0
control[d]	0	0	10	10
control	0		0	0

[a] One fish displayed a complete loss of equilibrium.

[b] Fish were lethargic.

[c] Mortality was judged to be toxicant related.

[d] This control set on 30 June with the 100% effluent solution.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-9 effluent and fathead minnow (*Pimephales promelas*)

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	5.3	8.9	7.9	7.6	7.2	7.1
	1.9	8.4	7.4	7.6	7.0	7.1
	0.41	7.6	7.4	6.9	7.0	7.1
	control	7.6	7.5	6.9	7.1	7.1
DO (mg/ℓ)	5.3	9.0(>100)[a]	0.4(4.5)	0.3(3.4)	0.3(3.4)	1.7(19)
	1.9	9.0(>100)	4.8(55)	1.5(17)	1.3(15)	1.0(11)
	0.41	8.9(>100)	6.3(72)	4.2(78)	3.8(43)	3.5(40)
	control	8.9(>100)	7.2(82)	5.7(65)	5.3(60)	5.3(60)
specific conductance (μmhos/cm)	5.3	294				
	1.9	200				
	0.41	163				
	control	151				
total hardness as $CaCO_3$ (mg/ℓ)	5.3	44				
	1.9	44				
	0.41	44				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	5.3	65				
	1.9	44				
	0.41	34				
	control	30				

[a] % of saturation at 22°C.

Table CE4 -- Concentrations tested and corresponding percentage mortalities of fathead minnow (*Pimephales promelas*) exposed to CTHF-9 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
5.3[a]	100[a]	100[a]	100[a]	100[a]
3.2[a]	20[a,b,c]	90[a,c,d]	90[a,b]	90[a,b]
1.9	0	0[e]	10	20
1.1	0[e]	0	10	10
0.68	0	10	10	10
0.41	0	0	10	10
control	0	0	0	0

[a] Solutions were cloudy.

[b] Fish displayed a dark coloration.

[c] Some fish displayed a complete loss of equilibrium.

[d] Fish were at the surface, gulping air.

[e] Fish were lethargic.

Table CE4-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-10 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	60	7.2	6.8	6.7	6.7	6.7
	22	7.3	7.0	6.7	6.6	6.8
	4.6	7.3	7.2	7.0	6.9	6.9
	control	7.2	7.0	6.8	6.9	6.9
DO (mg/ℓ)	60	8.3(94)[a]	0.3(3.4)	0.2(2.3)	0.1(1.1)	0.1(1.1)
	22	8.7(99)	4.1(47)	0.2(2.3)	0.2(2.3)	0.2(2.3)
	4.6	8.8(100)	7.1(81)	3.9(44)	3.7(42)	2.2(25)
	control	8.8(100)	7.5(85)	4.8(55)	4.4(50)	4.4(50)
specific conductance (μmhos/cm)	60	186				
	22	160				
	4.6	142				
	control	142				
total hardness as $CaCO_3$ (mg/ℓ)	60	30				
	22	36				
	4.6	42				
	control	42				
total alkalinity as $CaCO_3$ (mg/ℓ)		42				
		37				
		33				
		30				

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[a] % of saturation at 22°C.

Table CE4 -- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-10 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration[a] (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
60	100	100	100	100
36	100	100	100	100
22	90[b]	100	100	100
13	10[b]	100	100	100
7.8	0[c]	0[c]	0[c]	0
4.6	0	0	0[b,c]	10[c]
control	0	0	0	0

[a] All effluent solutions were cloudy in proportion to the concentration for the duration of the test.

[b] Some fish displayed a loss of equilibrium.

[c] Fish were lethargic.

Table CE2- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-11 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	100	7.7	7.1	6.6	6.5	6.8
	36	7.4	7.1	6.8	6.8	6.9
	7.8	7.3	7.2	6.9	6.9	6.8
	control	7.2	7.0	6.9	6.9	6.7
DO (mg/ℓ)	100	11(>100)[a]	7.4(84)	4.2(48)	1.9(22)	3.0(34)
	36	9.6(>100)	6.8(77)	3.9(44)	3.1(35)	3.6(41)
	7.8	8.9(>100)	7.2(82)	4.3(49)	4.0(45)	3.8(43)
	control	8.5(97)	6.6(75)	4.3(49)	4.2(48)	3.3(38)
specific conductance (μmhos/cm)	100	36				
	36	95				
	7.8	130				
	control	142				
total hardness as $CaCO_3$ (mg/ℓ)	100	1				
	36	28				
	7.8	40				
	control	42				
total alkalinity as $CaCO_3$ (mg/ℓ)	100	7				
	36	21				
	7.8	29				
	control	30				

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[a] % of saturation at 22°C.

Table CE4-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-11 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality 24-hour	48-hour	72-hour	96-hour
100	20[a]	40[a,b]	60[b,c]	60[a]
60	0	0	20	40[b,d]
36	0	0	0	0
22	0	0[b]	0[b]	10
13	0[b]	0[b]	0[b]	10
7.8	0	0	0	10
control	0	0	0	0

[a] Some fish displayed a loss of equilibrium.

[b] Some fish displayed a dark coloration.

[c] Fish were at the surface.

[d] Fish were lethargic.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-12 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	100	7.5	6.9	6.8	6.6	6.4
	46	7.4	7.0	6.8	6.8	6.9
	15	7.3	7.1	7.0	6.9	6.9
	control	7.2	7.0	7.0	6.9	6.7
DO (mg/ℓ)	100	11(>100)[a]	8.5(97)	6.8(77)	4.5(51)	2.8(32)
	46	9.5(>100)	7.7(88)	4.0(45)	4.0(45)	3.8(43)
	15	8.9(>100)	7.2(82)	4.7(53)	4.9(53)	4.7(53)
	control	8.6(98)	7.4(84)	5.5(63)	4.2(48)	4.2(48)
specific conductance (μmhos/cm)	100	28				
	46	95				
	15	130				
	control	142				
total alkalinity as $CaCO_3$ (mg/ℓ)	100	5				
	46	18				
	15	26				
	control	30				
total hardness as $CaCO_3$ (mg/ℓ)	100	1				
	46	24				
	15	36				
	control	42				

[a] % of saturation at 22°C.

Table CE3-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-12 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
100	10	10	10	10
68	10	20	20	20
46	0	0[a]	0[a]	0
32	10[a]	20	20[b]	30
22	10	10[ab]	10[ab]	20
15	0	0	0	0
Control	0	0	0	0

[a] Some fish displayed a dark coloration.

[b] Some fish displayed a loss of equilibrium.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-13 effluent and fathead minnow (Pimephales promelas).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour[a]
pH	10	7.4	7.1	7.0	7.0	-
	2.8	7.3	6.9	6.8	6.9	-
	0.41	7.2	7.1	6.8	6.9	-
	control	7.0	7.2	6.9	7.0	-
DO (mg/ℓ)	10	8.7(99)[b]	0.2(2.3)	0.2(2.3)	0.2(2.3)	-
	2.8	8.9(>100)	1.0(11)	0.3(3.4)	0.7(8.0)	-
	0.41	9.0(>100)	5.1(58)	2.4(27)	2.3(26)	-
	control	8.6(98)	6.6(75)	4.2(48)	4.0(45)	-
specific conductance (μmhos/cm)	10	293				
	2.8	185				
	0.41	148				
	control	142				
total hardness as $CaCO_3$ (mg/ℓ)	10	56				
	2.8	46				
	0.41	42				
	control	42				
total alkalinity as $CaCO_3$ (mg/ℓ)	10	63				
	2.8	41				
	0.41	34				
	control	30				

[a] Due to a scheduling oversight, pH and DO measurements were not made at 96-hours.

[b] % of saturation at 22°C.

Table CE4 -- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-13 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration[a] (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
10	100	100	100	100
5.3	100	100	100	100
2.8	100	100	100	100
1.5	0	30[b,c,d,e]	30[b,d]	40[b]
0.78	0	0	0	0
0.41	0	0	0	0
control	0	0	0	0

[a] All effluent test solutions were cloudy in proportion to the concentration for the duration of the test.

[b] Fish displayed a dark coloration.

[c] Fish were lethargic.

[d] Some fish were at the surface.

[e] Some fish were gulping at the surface.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-14 effluent and fathead minnow (*Pimephales promelas*).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	53	7.3	6.9	6.7	6.7	6.7
	19	7.5	7.2	6.8	6.8	6.8
	4.1	7.4	7.3	6.9	6.8	6.9
	control	7.2	7.1	6.9	6.9	7.0
DO (mg/ℓ)	53	7.1(81)[a]	2.3(26)	0.2(2.3)	0.3(3.4)	0.5(5.7)
	19	8.5(87)	6.1(69)	0.8(9.1)	1.2(14)	2.1(24)
	4.1	9.0(>100)	7.4(84)	3.1(35)	2.9(33)	3.4(39)
	control	8.8(100)	7.7(88)	4.8(55)	4.7(56)	5.4(61)
specific conductance (μmhos/cm)	53	471				
	19	265				
	4.1	183				
	control	142				
total hardness as $CaCO_3$ (mg/ℓ)	53	36				
	19	40				
	4.1	42				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	53	40				
	19	35				
	4.1	32				
	control	30				

[a] % of saturation at 22°C.

Table CE4-- Concentrations tested and corresponding percentage mortalities of fathead minnow (*Pimephales promelas*) exposed to CTHF-14 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
53	100	100	100	100
32	30	50[a,b]	100	100
19	0	0[b]	10	10
11	0	0	0	0
6.8	0	0	0	0
4.1	0	0	0	10[c]
control	0[d]	0	0	0

[a] Some fish displayed a loss of equilibrium.

[b] Some fish were at the surface.

[c] This mortality was judged not to be toxicant related.

[d] Fish were lethargic.

Table C-2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-15 effluent and fathead minnow (*Pimephales promelas*).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	100	8.2	7.4	7.2	7.2	7.0
	22	8.0	7.4	7.0	7.0	7.1
	4.6	7.9	7.3	7.0	7.0	7.2
	control	7.9	7.2	7.0	7.1	7.1
DO (mg/ℓ)	100	9.4(>100)[a]	7.6(86)	5.8(66)	4.4(50)	4.4(50)
	22	9.0(>100)	7.5(85)	4.8(55)	4.5(51)	4.9(56)
	4.6	9.0(>100)	7.6(86)	4.7(53)	4.9(56)	5.3(60)
	control	8.9(>100)	7.3(83)	4.7(53)	4.9(56)	5.5(63)
specific conductance (µmhos/cm)	100	100				
	22	143				
	4.6	150				
	control	150				
total hardness as $CaCO_3$ (mg/ℓ)	100	2				
	22	34				
	4.6	42				
	control	44				
total alkalinity as $CaCO_3$ (mg/ℓ)	100	16				
	22	27				
	4.6	30				
	control	30				

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[a] % of saturation at 22°C.

Table CE3-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-15 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
100	0	0	10	20
60	0	0	0[a,b,c]	0[d]
36	0	0	0	0
22	0	0	0	0[a]
13	0	0	0	0
7.8	0	0	0	0[d]
4.6	0	0	0	0
control	0	0	0	0

[a] Some fish were lethargic.

[b] Fish were at the surface.

[c] Fish were gulping.

[d] Fish displayed a dark coloration.

Table CE2-- The pH, DO, specific conductance, total hardness and alkalinity measurements made during a 96-hour toxicity determination with CTHF-16 effluent and fathead minnow (*Pimephales promelas*).

	Nominal concentration (%)	0-hour	24-hour	48-hour	72-hour	96-hour
pH	36	8.0	7.2	7.1	7.0	7.0
	3.6	7.6	7.1	7.0	7.0	6.9
	0.36	7.5	7.0	7.1	7.1	7.0
	control	7.5	7.0	7.2	7.2	7.1
DO (mg/ℓ)	36	9.7(>100)[a]	0.3(3.4)	0.4(4.5)	0.2(2.3)	0.2(2.3)
	3.6	9.3(>100)	6.1(69)	2.2(25)	1.2(14)	1.6(18)
	0.36	9.4(>100)	6.3(72)	3.3(38)	3.1(35)	2.8(32)
	control	9.1(>100)	6.2(70)	4.1(47)	4.1(47)	3.9(44)
specific conductance (μmhos/cm)	36	930				
	3.6	232				
	0.36	166				
	control	143				
total hardness as $CaCO_3$ (mg/ℓ)	36	-[b]				
	3.6	50				
	0.36	44				
	control	46				
total alkalinity as $CaCO_3$ (mg/ℓ)	36	58				
	3.6	35				
	0.36	33				
	control	32				

[a] % of saturation at 22°C.

[b] No measurement could be made due to similarity of effluent and titration end point color.

Table CE4-- Concentrations tested and corresponding percentage mortalities of fathead minnow (Pimephales promelas) exposed to CTHF-16 effluent for 24-, 48-, 72- and 96-hours.

Nominal concentration (%)	% mortality			
	24-hour	48-hour	72-hour	96-hour
36	100	100	100	100
17	100	100	100	100
7.8	10	100	100	100
3.6	10	20	20	20
1.7	0	0	0[b]	0[b]
0.78	0[a]	0	0[b]	0[b]
0.36	0	0	0[b]	0[b]
control	0	0	0	0

[a] Some fish displayed a dark coloration.

[b] Fish were lethargic.

APPENDIX CF

WATER QUALITY ANALYSIS OF THE 14 WASTEWATER SAMPLES AS A FUNCTION OF TEST SOLUTION CONCENTRATIONS AND RAW MORTALITY DOSE RESPONSE DATA AS DIRECTLY REPORTED BY EG&G BIONOMICS FOR DAPHNIA ACUTE TOXICITY TESTS

TABLE CF1 -- Water quality analysis of CTHF-3 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved oxygen[a] (mg/ℓ)	pH[a]	Total hardness[b] (mg/ℓ $CaCO_3$)	Specific conductance[b] (µmhos/cm^2)	Alkalinity[b] (mg/ℓ $CaCO_3$)
100	9.1-4.6	9.3-8.2	28	648	143
36	9.0-7.1	8.8-8.3	140	668	136
13	8.7-7.5	8.3-8.3	196	711	134
control	8.8-8.5	8.1-8.4	202	669	135

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (Daphnia magna) exposed to CTHF-3 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
100	93	100
60	73	93
36	0	87
22	0	20
13	0	13
control	0	0

TABLE CF1 -- Water quality analysis of CTHF-4 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved[a] oxygen (mg/ℓ)	pH[a]	Total[b] hardness (mg/ℓ $CaCO_3$)	Specific[b] conductance (μmhos/cm²)	Alkalinity[b] (mg/ℓ $CaCO_3$)
100	12.0-8.0	7.6-7.5	6	30	8
36	9.6-8.1	8.1-8.2	138	410	94
7.8	9.2-8.2	8.2-8.2	196	590	128
control	8.9-8.1	8.1-8.2	212	610	141

[a] Measurements taken at 0- and 48-hour.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-4 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	 48-hour
100	93	100
60	0	67
36	0	7
22	0	0
13	0	0
7.8	0	0
control	0	0

TABLE CF1 -- Water quality analysis of CTHF-5 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved oxygen (mg/ℓ)[a]	pH[a]	Total hardness (mg/ℓ $CaCO_3$)[b]	Specific conductance (µmhos/cm^2)[b]	Alkalinity (mg/ℓ $CaCO_3$)[b]
100	8.8-8.7	9.3-7.8	4	249	7
36	8.9-8.5	8.7-7.8	136	460	86
13	8.8-8.6	8.4-7.6	184	590	126
control	8.9-8.7	8.2-8.1	214	600	126

[a] Measurements taken at 0- and 48-hour.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-5 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
100	100	100
60	27	93
36	0	13
22	0	7
13	0	0
control	0	0

TABLE CF1 -- Water quality analysis of CTHF-6 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved[a] oxygen (mg/ℓ)	pH[a]	Total[b] hardness (mg/ℓ $CaCO_3$)	Specific[b] conductance (μmhos/cm^2)	Alkalinity[b] (mg/ℓ $CaCO_3$)
13	8.9-4.3	9.0-8.3	206	1165	260
4.6	8.9-5.7	8.7-8.2	226	821	178
1.5	8.6-6.9	8.5-8.1	204	762	149
control	8.8-8.5	8.1-8.4	202	669	135

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (Daphnia magna) exposed to CTHF-6 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality	
	24-hour	48-hour
13	71[a]	100[a]
7.8	27	87
4.6	0	53
2.8	0	0
1.5	0	0
control	0	0

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[a] Data based on 14 daphnids. One daphnid could not be accounted for.

TABLE CF1 -- Water quality analysis of CTHF-7 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved oxygen[a] (mg/ℓ)	pH[a]	Total hardness[b] (mg/ℓ $CaCO_3$)	Specific conductance[b] (µmhos/cm²)	Alkalinity[b] (mg/ℓ $CaCO_3$)
100	9.2-2.5	9.7-8.1	14	1000	239
36	9.0-4.4	9.0-8.0	136	801	172
13	8.8-6.3	8.6-8.0	192	780	147
control	8.8-8.5	8.1-8.4	202	669	135

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken at 0-hour.

TABLE CF2-- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-7 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
100	47	100
60	0	100
36	0	73
22	0	33
13	0	13
control	0	0

TABLE CF1 -- Water quality analysis of CTHF-8 effluent test solutions during the static acute exposure of the water flea (Daphnia magna).

Nominal concentration (%)	Dissolved[a] oxygen (mg/ℓ)	pH[a]	Total[b] hardness (mg/ℓ $CaCO_3$)	Specific[b] conductance (μmhos/cm^2)	Alkalinity[b] (mg/ℓ $CaCO_3$)
100	9.7-6.7	8.2-8.3	2	41	109
36	9.2-7.3	8.2-8.1	142	400	122
13	9.0-7.9	8.2-7.9	186	550	126
control	8.9-8.7	8.2-8.1	214	600	126

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (Daphnia magna) exposed to CTHF-8 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	Average percentage mortality 48-hour
100	0	13
60	0	0
36	0	0
22	0	0
13	0	0
control	0	0

TABLE CF1 -- Water quality analysis of CTHF-9 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved oxygen[a] (mg/ℓ)	pH[a]	Total hardness[b] (mg/ℓ $CaCO_3$)	Specific conductance[b] (µmhos/cm²)	Alkalinity[b] (mg/ℓ $CaCO_3$)
19	9.2-0.7	8.9-8.9	190	1000	239
6.8	9.0-5.1	8.6-8.2	198	750	179
1.5	8.9-7.1	8.2-8.2	212	610	145
control	8.9-8.1	8.1-8.2	212	610	141

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-9 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	 48-hour
19	87	93
11	33	53
6.8	7	27
4.1	0	0
2.4	0	0
1.5	0	0
control	0	0

TABLE CF1-- Concentrations tested and corresponding average observed percentage mortalities for the water flea (Daphnia magna) exposed to CTHF-10 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
100	33	100
60	27	93
36	0	87
22	0	0
13	0	0
control	0	0

TABLE CF1 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-11 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	Average percentage mortality 48-hour
100	0	93
60	0	0
36	0	0
22	0	0
13	0	0
control	0	0

TABLE CF1-- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-12 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	Average percentage mortality 48-hour
100	100	100
60	0	0
36	0	0
22	0	0
13	0	0
control	0	0

TABLE CF1-- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-13 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
13	67	100
7.8	13	87
4.6	0	87
2.8	0	0
1.7	0	0
control	0	0

TABLE CF1 -- Water quality analysis of CTHF-14 effluent test solutions during the static acute exposure of the water flea (Daphnia magna).

Nominal concentration (%)	Dissolved[a] oxygen (mg/ℓ)	pH[a]	Total[b] hardness (mg/ℓ $CaCO_3$)	Specific[b] conductance (µmhos/cm²)	Alkalinity[b] (mg/ℓ $CaCO_3$)
100	9.0-4.7	7.0-7.5	38	858	40
36	7.5-7.1	7.8-8.1	148	803	94
13	8.3-7.5	7.9-8.2	194	797	122
control	8.8-8.5	8.1-8.4	202	669	135

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken a 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-14 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
100	60	100
60	0	93[a]
36	0	0[a]
22	0	7[a]
13	0	0[a]
control	0	0

---

[a] Some surviving daphnids were lethargic.

TABLE CF1-- Water quality analysis of CTHF-15 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved[a] oxygen (mg/ℓ)	pH[a]	Total[b] hardness (mg/ℓ $CaCO_3$)	Specific[b] conductance (μmhos/cm²)	Alkalinity[b] (mg/ℓ $CaCO_3$)
100	9.0-7.7	8.0-8.2	2	138	16
36	9.0-7.7	8.1-8.1	154	524	99
13	8.9-7.5	8.1-8.1	184	672	118
control	8.8-8.5	8.1-7.9	202	669	135

[a] Measurements taken at 0- and 48-hours.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-15 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	48-hour
100	47	87
60	0[a]	7[a]
36	0[a]	0[a]
22	0[a]	0[a]
13	0[a]	0[a]
control	0	0

[a] Some surviving daphnids became entrapped at the air-water interface.

TABLE CF1 -- Water quality analysis of CTHF-16 effluent test solutions during the static acute exposure of the water flea (*Daphnia magna*).

Nominal concentration (%)	Dissolved oxygen[a] (mg/ℓ)	pH[a]	Total hardness[b] (mg/ℓ $CaCO_3$)	Specific conductance[b] (µmhos/cm^2)	Alkalinity[b] (mg/ℓ $CaCO_3$)
36	9.0-4.7	8.1-7.7	292	1292	122
7.8	8.7-8.0	8.1-8.0	224	821	132
1.7	8.6-8.5	8.1-8.1	218	716	134
control	8.6-8.6	7.8-8.0	208	662	132

[a] Measurements taken at 0- and 48-hour.

[b] Measurements taken at 0-hour.

TABLE CF2 -- Concentrations tested and corresponding average observed percentage mortalities for the water flea (*Daphnia magna*) exposed to CTHF-16 effluent. Each mortality value represents the average of 3 replicates.

Nominal concentration (%)	Average percentage mortality 24-hour	Average percentage mortality 48-hour
36	33	100
22	7	47
13	0	13
7.8	0	20
4.6	0	20
2.8	0	0
1.7	0	0
control	0	7

APPENDIX CG

RAW DATA ON ACUTE ORAL TOXICITY STUDY IN RATS PERFORMED BY LITTON BIONETICS, INC.

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-3

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-01

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-3
Test B-2
Date 6-12-78
Time 10:30

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2891.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 172 to 226 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
Males					
0	216	265	343	-	0/5
10	213	294	360	-	0/5
Females					
0	178	190	231	-	0/5
10	178	216	239	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:	Reviewed by:	
David R. Damske	Robert P Beliles	9/8/74
David R. Damske, B.A. Toxicology Technician Department of Toxicology	Robert P. Beliles, Ph.D. Director Department of Toxicology	Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-4

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-02

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-4
Test B-2
Date 6-12-78
Time 1500

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2892.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 168 to 214 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
Males					
0	216	265	343	-	0/5
10	206	288	348	-	0/5
Females					
0	178	190	231	-	0/5
10	184	219	242	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals. The lungs of two treated females were observed to be slightly mottled. This finding has previously been observed in this strain of animal in this laboratory and was judged not to be related to compound administration.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R Damske
David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P Beliles
Robert P. Beliles, Ph.D.
Director
Department of Toxicology

9/8/94
Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-5

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-03

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-5
Test Sample B-2
Date 6-12-78
Time 1530

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2893.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 166 to 223 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	214	305	364	-	0/5
		Females			
0	178	190	231	-	0/5
10	179	217	244	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals. An enlarged, pitted, fluid-filled right kidney was observed in one treated male rat. This finding has previously been observed in this strain of animal at this laboratory and was judged not to be related to compound administration.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P Beliles

Robert P. Beliles, Ph.D.
Director
Department of Toxicology

9/4/78
Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-6

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-04

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-6
Test B-2
Date 6-12-78
Time 1400

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2894.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 164 to 225 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided ad libitum with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	211	299	356	-	0/5
		Females			
0	178	190	231	-	0/5
10	175	216	240	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P Beliles
Robert P. Beliles, Ph.D.
Director
Department of Toxicology

9/8/78
Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-7

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-05

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-7
Test B-2
Date 6-13-78
Time 0900

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2895.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 179 to 220 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle Water and Purina Laboratory Chow were provided _ad libitum_ with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
			Males		
0	216	265	343	-	0/5
10	207	292	343	-	0/5
			Females		
0	178	190	231	-	0/5
10	189	222	247	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P Beliles

Robert P. Beliles, Ph.D.
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Department of Toxicology

9/8/78
Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-8

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-06

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-8
Test B-2
Date 6-17-78
Time 2300

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2896

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 156 to 233 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided ad libitum with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	208	285	335	-	0/5
		Females			
0	178	190	231	-	0/5
10	178	216	240	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R. Damske

David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P. Beliles 9/8/78

Robert P. Beliles, Ph.D.
Director
Department of Toxicology

Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-9

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-07

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-9
Test B-2
Date 6-17-78
Time 2300

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2897.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 166 to 246 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided ad libitum with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
	Males				
0	216	265	343	-	0/5
10	212	261	321	-	0/5
	Females				
0	178	190	231	-	0/5
10	179	223	242	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals. One treated female was noted to have an enlarged, fluid-filled kidney; the cortex was not solid. This finding has previously been observed in this strain of animal in this laboratory and was judged not to be related to compound administration.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P. Beliles 9/8/78

Robert P. Beliles, Ph.D.
Director
Department of Toxicology

Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-10

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-08

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-10
Test B-2
Date 6-2-78
Time 1200

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2898.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 155 to 252 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	224	301	358	-	0/5
		Females			
0	178	190	231	-	0/5
10	169	199	215	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:

David R Damske
David R. Damske, B.A.
Toxicology Technician
Department of Toxicology

Reviewed by:

Robert P Beliles
Robert P. Beliles, Ph.D.
Director
Department of Toxicology

9/8/78
Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-11

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-09

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-11
Test B-2
Date 6-2-78
Time 1300

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2899.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 178 to 229 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided ad libitum with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	214	301	359	-	0/5
		Females			
0	178	190	231	-	0/5
10	186	220	244	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

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Reviewed by:

Robert P Beliles 9/8/78

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Director
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Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-12

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-10

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-12
Test Sample B-2
Date 6-2-78
Time 1300

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2900.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 157 to 239 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided _ad libitum_ with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	222	305	361	-	0/5
		Females			
0	178	190	231	-	0/5
10	184	226	245	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

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9/8/78
Date

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-13

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-11

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-13
Test B-2
Date 6-2-78
Time 1300

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2901.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 161 to 218 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided ad libitum with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	209	280	336	-	0/5
		Females			
0	178	190	231	-	0/5
10	184	217	238	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals. The uterus of one treated female was noted to be distended. This finding has been previously observed in this strain of animal in this laboratory and was judged not to be related to compound administration.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:	Reviewed by:	
David R. Damske	Robert P Beliles	9/18/78
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SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-14

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-12

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-14
Test B-2
Date 6-9-78
Time 1500

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2902.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 175 to 222 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
		Males			
0	216	265	343	-	0/5
10	217	308	364	-	0/5
		Females			
0	178	190	231	-	0/5
10	185	230	248	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values greater than 10 ml/kg were estimated for both male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

Submitted by:	Reviewed by:	
David R Damske	Robert P Beliles	9/8/78
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SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-15

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-13

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-15
Test B-3
Date 6-12-78
Time 0730

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2903.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 167 to 228 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day 0	7	14	Deaths Day 0-14	Total Mortality Deaths/Treated
Males					
0	216	265	343	-	0/5
10	214	307	360	-	0/5
Females					
0	178	190	231	-	0/5
10	183	204	234	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

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Reviewed by:

Robert P Beliles 9/4/74

Robert P. Beliles, Ph.D. Date
Director
Department of Toxicology

SPONSOR: Monsanto Research Corporation

MATERIAL: CTHF-16

SUBJECT: FINAL REPORT
Acute Oral Toxicity Study in Rats
LBI Project No. 20969-14

1. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the test compound when administered by oral gavage to male and female rats.

2. MATERIAL

A glass bottle containing one liter of a liquid labeled:

CTHF-16
Test B-2
Date 6-12-78
Time 0730

was received from Clemson University by Litton Bionetics, Inc. (LBI) on June 20, 1978 and designated as LBI No. 2904.

3. EXPERIMENTAL DESIGN

Young adult rats (weighing 180 to 219 g and eight to nine weeks of age at the time of treatment, July 26, 1978) of the Charles River CD strain [CRL:COBS CD (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for six days. The animals were individually housed in wire-bottom cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Water and Purina Laboratory Chow were provided *ad libitum* with the exception of the night before treatment when food was removed from the cages.

The test material was given undiluted. A single dose (10 ml/kg) of the test material was administered by oral gavage to five rats of each sex. A group of 10 untreated rats (five of each sex) served as a control for all materials tested in this project (LBI Project Nos. 20969-01 through -14).

The rats were observed frequently on the day of treatment and daily thereafter. The animals were weighed on the day of treatment and on Days 7 and 14 following treatment. Necropsies were performed on the surviving animals killed 14 days after treatment.

4. RESULTS

The data have been summarized as follows.

Dose (ml/kg)	Mean Body Weight (g) Day			Deaths Day	Total Mortality
	0	7	14	0-14	Deaths/Treated
			Males		
0	216	265	343	-	0/5
10	209	293	352	-	0/5
			Females		
0	178	190	231	-	0/5
10	187	213	250	-	0/5

Based on the absence of deaths in the 14 days following treatment, LD50 values of greater than 10 ml/kg were estimated for male and female rats.

No signs of toxicity or abnormal necropsy findings were observed in any of the treated or control animals.

5. CONCLUSION

Following the oral administration of a single dose (10 ml/kg) of the test compound to fasted young adult rats, no mortalities were observed. Therefore, the median lethal dose was judged to be greater than 10 ml/kg.

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9/8/78
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CONVERSION FACTORS AND METRIC PREFIXES (15)

CONVERSION FACTORS

To convert from	To	Multiply by
Degree Celsius (°C)	Degree Fahrenheit (°F)	$t_{°F} = 1.8\, t_{°C} + 32$
Grams/meter^3 g/m^3)	Milligrams/liter	1.0
Kilogram (kg)	Pound-mass (avoirdupois)	2.205
Meter (m)	Inch	3.937×10^1
Meter^3 (m^3)	Gallon (U.S. liquid)	2.642×10^2
Meter^3 (m^3)	Liter	1.0×10^3

METRIC PREFIXES

Prefix	Symbol	Multiplication factor	Example
Kilo	k	10^3	5 kg = 5 x 10^3 grams
Centa	c	10^{-2}	5 cm = 5 x 10^{-2} meters
Milli	m	10^{-3}	5 mg = 5 x 10^{-3} gram
Micro	μ	10^{-6}	5 μg = 5 x 10^{-6} gram

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(15) Standard for Metric Practices. ANSI/ASTM Designation: E 380-76$^{\epsilon}$, IEEE Std 268-1976, American Society for Testing and Materials, Philadelphia, Pennsylvania, February 1976. 37 pp.

APPENDIX D

HYPERFILTRATION OF NONELECTROLYTES: DEPENDENCE OF REJECTION ON SOLUBILITY PARAMETERS

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SUMMARY

The dependence of hyperfiltration rejection of nonelectrolyte solutes in single-solute water solutions on solubility parameters is demonstrated using hyperfiltration results reported in the literature. The hyperfiltration systems are characterized by a solubility parameter derived empirically from the rejection-solubility parameter dependence. A criterion for high rejection follows.

INTRODUCTION

Hyperfiltration possesses high potential for separating toxic solutes in industrial unit operation effluents (1). Some of the nonelectrolytes of concern are quite volatile and many are only slightly soluble in water. Thus, the direct experimental measurement of the salt rejection $\underset{\sim}{R}_i$ of the approximately 100 nonelectrolyte priority pollutants would be difficult and a reliable method for predicting $\underset{\sim}{R}_i$ in a hyperfiltration system from a few reference measurements and molecular properties of the solutes i would be valuable.

The most detailed model developed for this purpose has been provided by Sourirajan and coworkers (2). Using one or more molecular properties (acidity, basicity, Hammett and Taft numbers, steric parameters and Small's number) and the permeability and rejection of a reference solution one can relate these properties to $\underset{\sim}{R}_i$. Other models use flux equations to relate the measurable properties of a hyperfiltration system (3, 4). All approaches include both a transport property of the hyperfiltration system and a coefficient for the distribution of the solute between the bulk solution and the barrier.

We have previously pointed out the value of solute molecular weights in predicting $\underset{\sim}{R}_i$ of nonelectrolytes (1). Most high rejection hyperfiltration membranes effectively reject nonelectrolytes with molecular weights greater than about 80. Cellulose acetate is an exception to this generalization. Although scatter can be large in plots of rejection vs. molecular weights, when the molecular weight is the most reliable or perhaps the only molecular property available it can be used to estimate $\underset{\sim}{R}_i$ in systems characterized by a few measurements.

This report demonstrates a dependence of $\underset{\sim}{R}_i$ for nonelectrolytes on the solute solubility parameter introduced by Hildebrand and Scott (5) and characterizes the hyperfiltration system by solubility parameters. It also provides an empirical method for the rough estimation of $\underset{\sim}{R}_i$ for a solute of known solubility parameter in a hyperfiltration system from values of $\underset{\sim}{R}_i$ obtained for a few reference solutes without explicitly considering a transport property for the solute, providing its molecular volume is not vastly larger than those of the reference solutes.

Chian and Fang (6) proposed the difference between the solubility parameters of the solute and membrane plays the major role in determining $\underset{\sim}{R}_i$ of nonelectrolyte solutes. Klein _et_ _al_. (3) qualitatively related solute permeabilities in the absence of hydraulic flux with two-dimensional solubility parameters and used the experimental permeabilities to predict specific separations of organic solutes under hyperfiltration conditions. A quantitative correlation of $\underset{\sim}{R}_i$ with solubility parameters was not attempted in either report.

DEFINITIONS, CONCEPTS, AND CALCULATIONS

The solubility parameter is defined by $\delta \equiv (\Delta\bar{E}/\bar{V})^{1/2}$ where $\Delta\bar{E}/\bar{V}$ is the energy of evaporation per unit volume, called the cohesive energy density. The units of δ are $(J/m^3)^{1/2}$. Solubility parameter theory predicts that the best solvent for a given solute, e.g. a polymer, is one whose solubility parameter is equal to or close to that of the solute (5).

The rejection R_i of a solute is defined as $1 - C_p/C_b$, where C_p and C_b are concentrations in the permeate and bulk feed solutions respectively. An intrinsic rejection, $1 - C_p/C_w$ based on the concentrations of permeate and that occurring at the feed-membrane interface C_w is commonly defined. Normally the intrinsic rejection is projected as the infinite-velocity asymptote of the rejection $\underset{\sim}{R}_i$, and this intrinsic rejection is the property logically addressed in this study. Because most investigations are conducted so as to preclude large differences in the two rejections, and virtually no data exist projecting the intrinsic rejection, the observed rejection is used throughout. Errors produced by this simplification may be substantial and are largest near rejection of 0.5.

In hyperfiltration the distribution of solute between the bulk solution and the barrier is assumed to be important in determining $\underset{\sim}{R}_i$. Further, assuming the concentration of solute available for transport across the hyperfilter depends on $\Delta_{im} = \delta_i - \delta_m$, where δ_m characterizes the hyperfilter, $\underset{\sim}{R}_i$ should be a function of Δ_{im}. Of course, an attempt to relate $\underset{\sim}{R}_i$ to Δ_{im} alone is imcomplete because a transport property characterizing the hyperfiltration system is not explicitly included.

The group contribution method of Konstram and Fairheller (5) was used to calculate Small's number (8) S_i and the δ_i were obtained by $\delta_i = S_i/\bar{v}_i$, where $\bar{v}_i$ is the molar volume. This method is used although tables containing δ_i for many solutes are available (5, 9). It is desirable to use a consistent method for as many compounds as possible. Even with the use of this general approach some values of δ_i are not available, especially those for polyfunctional molecules. Values of $\bar{v}_i$ were calculated by dividing molecular weight of the solute M_i by its density ρ_i in the liquid state at the temperature of the experiment, with M_i and ρ_i obtained from commonly used tables (10).

DEPENDENCE OF REJECTION ON SOLUBILITY PARAMETERS

Figures 1 - 5 show the dependence of $\underset{\sim}{R}_i$ on δ_i for the six hyperfiltration systems described in Table 1 (11 - 15). The δ_i occurring at $\underset{\sim}{R}_i = 0$ is assumed to be the solubility parameter characterizing the membrane system and is designated δ_m. The δ_m were obtained by a visual linear extrapolation of the plots. A slope of -10×10^{-3} $(m^3/J)^{1/2}$ was satisfactory for all graphs in the region $\Delta_{im} < 0$. Insufficient data are provided to determine δ_m by extrapolation in the poly(ether/amide) thin film composite (PA-300) systems, Figure 4. Using the slope observed in the remainder of the graphs, δ_m should be in the interval $34 \times 10^3 < \delta_m < 36 \times 10$ $(J/m^3)^{1/2}$. It should be noted that the scatter is very large in the cellulose acetate systems.

In the Permasep B-9 and cellulose acetate systems, Figures 2 and 5, several values of $\underset{\sim}{R}_i$ at $\Delta_{im} > 0$ are available. It is clear in Figure 2 that $\underset{\sim}{R}_i$ increases monotonically with increasing Δ_{im} in the region $\Delta_{im} > 0$. The dependence is not well defined in the cellulose acetate case where in this region several of the solutes listed contain two functional groups and the calculations of δ_i is less reliable.

The molecular weight, or $\bar{v}_i$, may be used to estimate $\underset{\sim}{R}_i$ in many systems, however this dependence appears to be absent in the cellulose acetate system. Figure 6 is provided to illustrate this observation and it should be compared with Figure 5, where $\underset{\sim}{R}_i$ are plotted vs. δ_i.

DISCUSSION

The dependence of $\underset{\sim}{R}_i$ on δ_i has been illustrated in several hyperfiltration systems. It is evident that high rejection occurs when $|\Delta_{im}|$ is large. Figure 7 provides a graph of $\underset{\sim}{R}_i$ vs. Δ_{im} for all the membranes other than the cellulose acetate membranes and Figure 5 provides a graph of $\underset{\sim}{R}_i$ vs. δ_i for the cellulose acetate systems, where the scatter is greater.

This empirical treatment of rejection of nonelectrolytes in hyperfiltration systems also provides a method for estimating $\underset{\sim}{R}_i$ for any solutes of known δ_i from a few reference experiments. The value of δ_m is determined from a graph of $\underset{\sim}{R}_i$ vs. δ_i obtained for the reference solutes. Solutes having $\Delta_{im} < -10 \times 10^3$ $(J/m^3)^{1/2}$ should have $\underset{\sim}{R}_i > 0.90$. In the region $-10 \times 10^3 < \Delta_{im} < 0$ $(J/m^3)^{1/2}$, $\underset{\sim}{R}_i = -10 \times 10^{-3} \Delta_{im}$, this estimate being better for membranes other than cellulose acetate than for cellulose acetate.

The value of δ_m appears to characterize the hyperfiltration system with respect to its rejection of nonelectrolytes and provides an attractive criterion for selecting membranes for hyperfiltration applications. Although the nonelectrolyte priority pollutants possess a broad range of solubility parameters, the maximum appears to be about 28×10^3 $(J/m^3)^{1/2}$ and many are much smaller. Hyperfiltration systems with δ_m greater than about 36×10^3 or 38×10^3 $(J/m^3)^{1/2}$ should provide high rejections of these solutes.

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Table D1 -CHARACTERISTICS OF HYPERFILTRATION SYSTEMS

Membrane	Pressure (MPa)	Temperature (°C)	$10^{-3}\ \delta_m$ $(J/m^3)^{1/2}$	Classes of Solutes	Reference
Aromatic polyamide	1.72	25	29.0	alcohols, aldehyes, ethers, ketones	11
Aromatic polyamide, Permasep B-9, hollow fiber	3.10	20	30.0	alcohols, acids	12
Cellulose acetate	1.72	25	25.0	alcohols, aldehydes	11
Cellulose acetate	1.72	23-25	25.0	alcohols, aldehydes, esters, ethers, hydrocarbons	15
NS-100	5.52	25	34.5	acids, alcohols, aldehydes, esters, ketones, amines	13
Poly(ether/amide) PA-300	6.89	25	(~34)*	acid, alcohols, aldehydes, esters, ketone, chlorohydrocarbons	14

* Not enough data for extrapolation.

Refinement of this approach using solubility parameters as a measure of the membrane-solute interaction will likely require incorporation in a flux model.

ACKNOWLEDGEMENT

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REFERENCES

1. H. G. Spencer, J. L. Gaddis, and C. A. Brandon, Membranes for Toxic Control, presented at the Membrane Separation Technology Seminar, Clemson University, Clemson, SC 1977.

2. Summary of the method: S. Sourirajan and T. Matsuura, in "Reverse Osmosis and Synthetic Membranes," S. Souriragan, ed., National Research Council of Canada Publications, Ottawa, Canada, 1977, Chapter 2.

3. Examples: K. S. Spiegler and O. Kedem, *Desalination*, **1** (1966) 311; H. K. Lonsdale, U. Merten, and R. L. Riley, *J. Appl. Polymer Sci.*, **9** (1965) 1341; and L. Dresner and J. S. Johnson, Jr., in "Principles of Desalination," 2nd ed., K. S. Spiegler and A. D. K. Laird, eds., Academic Press, New York (in press).

4. E. Klein, J. Eichelberger, C. Eyer, and J. Smith, *Water Res.*, **9** (1975) 807.

5. J. H. Hildebrand and R. L. Scott, "The Solubility of Non-electrolytes," Rheinhold, New York, 1950.

6. E. S. K. Chian and H. H. P. Fang, AIChE Symposium Ser., **70** (1973) 497.

7. H. H. Konstam and W. R. Fairheller, Jr., *AIChE J*, **16** (1970) 837.

8. P. A. Small, *J. Appl. Chem.*, **3** (1953) 71.

9. J. L. Gordon, in *Encyclopedia of Polymer Science and Technology*, **3** (1965) 833; H. Burrell, *J. Paint Technol.*, **27** (1955) 726; C. M. Hansen, *Ind. Eng. Chem., Prod. Res. Dev.*, **8** (1960) 2.

10. "Handbook of Chemistry and Physics," R. E. Weast, ed., CRC Press, Inc., Cleveland, Ohio.

11. J. M. Dickson, T. Matsuura, P. Blais, and S. Sourirajan, *J. Appl. Polymer Sci.*, **19** (1975) 801.

12. V. B. Caracciolo, N. W. Rosenblatt, and V. J. Tomsic, in "Reverse Osmosis and Synthetic Membranes," S. Sourirajan, ed., National Research Council of Canada, Ottawa, Canada, 1977, Chapter 16.

13. L. T. Rozelle, J. E. Cadotte, K. E. Cobian, and C. V. Kopp, Jr., *ibid.*, Chapter 12.

14. R. L. Riley, R. L. Fox, C. R. Lyons, C. E. Milstead, M. W. Seroy, and M. Togami, Spiral-wound Poly (ether/amide) Thin-film Composite Membrane Systems, presented at the Membrane Separation Technology Seminar, Clemson University, Clemson, SC 1976.

15. T. Matsuura and S. Sourirajan, *J. Appl. Polymer Sci.*, **15**, 2905 (1971); *ibid.*, **16**, 1663, 2531 (1972); *ibid.*, **17**, 1043, 3683 (1973).

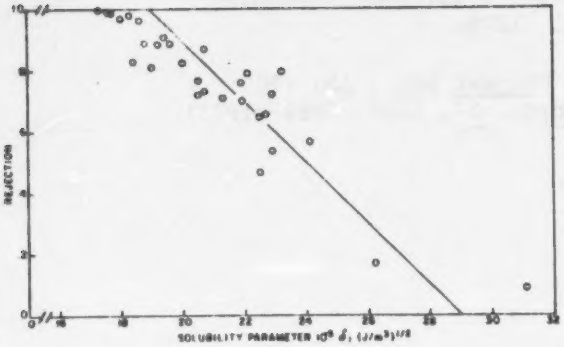


Figure D1 Solute rejection vs. solubility parameter: Polyamide membrane, 1.72 MPa, 25°C (11).

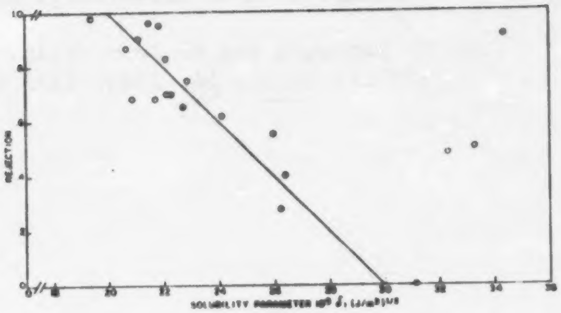


Figure D2 Solute rejection vs. solubility parameter: Polyamide, Permasep B-9, 3.10 MPa, 20°C (12).

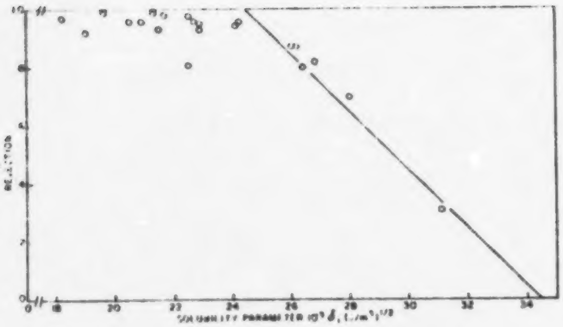


Figure **D3** Solute rejection vs. solubility parameter: NS-100, 5.52 MPa, 25°C (13).

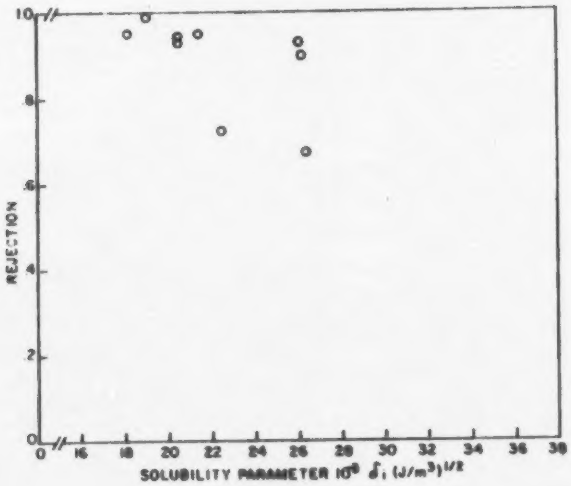
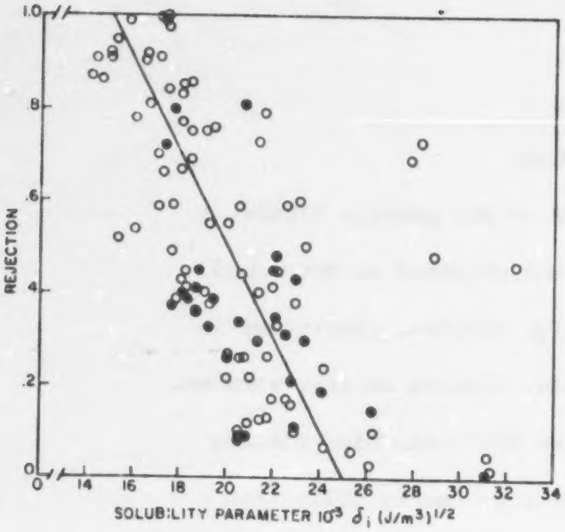


Figure **D4** Solute rejection vs. solubility parameter: poly(ether/amide), PA-300, 6.89 MPa, 25°C (14).



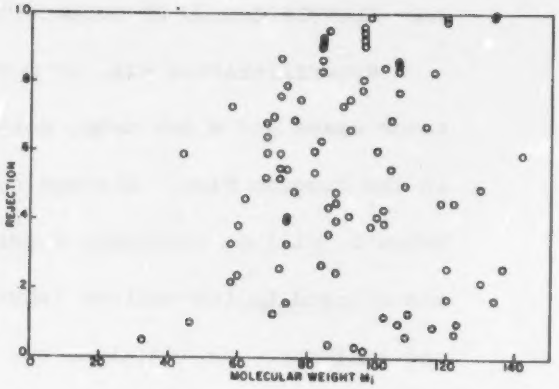


Figure **D5** Solute rejection vs. solubility parameter: cellulose acetate, 1.72 MPa, 23-25°C; O - (15), ● - (11).

Figure **D6** Solute rejection vs. molecular weight: cellulose acetate, 1.72 MPa, 23-25°C (15).

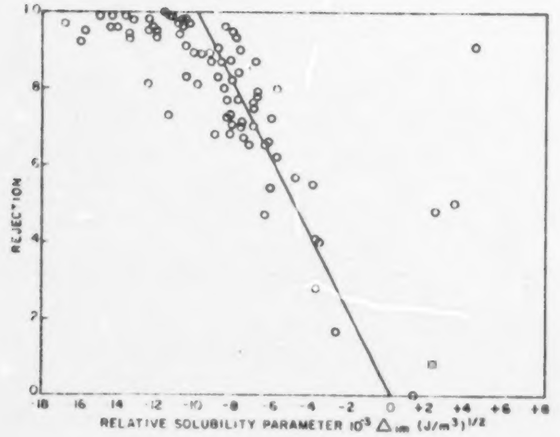


Figure **D7** Solute rejection vs. relative solubility parameter $\Delta_{im} = \delta_i - \delta_m$ for the noncellulosic membranes.

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APPENDIX E

1.0 IDENTIFICATION OF COLLECTION SAMPLES

Hyperfiltration will be performed on two process fluids, a scour waste and a dye drop, selected and obtained as described in the Program Plan. Sixteen collection samples, identified in Table 1, will be obtained, separated and bottled as test samples, and shipped to the various laboratories designated for testing and analysis. In addition, one-gallon contingency collection samples will be obtained and stored at Clemson University until the project is completed.

Table E1. COLLECTION SAMPLES FOR BIOASSAY TESTS AND CHEMICAL ANALYSES

Sample	Description	Volume (gallons)
CTHF-1	Plant water	5
CTHF-2	Apparatus water	5
CTHF-3	Scour-1, feed for PEA and CA hyperfiltration	25
CTHF-4	Scour-1, permeate from PEA hyperfiltration	25
CTHF-5	Scour-1, permeate from CA hyperfiltration	25
CTHF-6	Scour-1, concentrate from PEA and CA hyperfiltration	10[a]
CTHF-7	Scour-2, feed for DM hyperfiltration	25
CTHF-8	Scour-2, permeate from DM hyperfiltration	25
CTHF-9	Scour-2, concentrate from DM hyperfiltration	10[a]
CTHF-10	Dye-1, feed for PEA and CA hyperfiltration	25
CTHF-11	Dye-1, permeate from PEA hyperfiltration	25
CTHF-12	Dye-1, permeate from CA hyperfiltration	25
CTHF-13	Dye-1, concentrate from PEA and CA hyperfiltration	10[a]
CTHF-14	Dye-2, feed for DM hyperfiltration	25
CTHF-15	Dye-2, permeate from DM hyperfiltration	25
CTHF-16	Dye-2, concentrate from DM hyperfiltration	10[a]

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[a] Concentrate samples will be 2 - 5 gallons, containing equivalent solids to the feed sample.

2.0 BIOASSAY TESTS AND CHEMICAL ANALYSES

The planned bioassay tests and chemical analyses for the 129 consent decree priority pollutants are listed in Table 2. This table includes the test-sample container requirements and designates the collection samples for which each test is planned.

Table E2. BIOASSAY TESTS AND CHEMICAL ANALYSES, TEST-SAMPLE CONTAINERS, AND TESTS DESIGNATED FOR COLLECTION SAMPLES

Test #	Description	Sample Volume	Container	Required for Collection Samples (CTHF-)
B.1	Microbial mutogenicity (Ames) and cytotoxicity (hamster ovary cells)	500 mℓ	amber glass, Teflon-lined caps	3 - 16
B.2	Acute toxicity (rat)	500 mℓ	glass, Teflon-lined caps	3 - 16
B.3	Freshwater static bioassay (Daphnia and Fathead minnows)	20 gals[a]	5 gallon, plastic cubitainers	3 - 16
C.1	Volatile solutes	2 x 40 mℓ	glass vials, Teflon-lined septa	1 - 16
C.2	Nonvolatile solutes	2 x 1 gal	amber glass, Teflon-lined caps	1 - 16
C.3	Metals	500 mℓ	plastic bottles	1 - 16
C.4	Cyanide	500 mℓ	plastic bottles	1 - 16
C.5	Phenols	500 mℓ	amber glass	1 - 16
C.6	Pesticides	(use part of test sample C.2)		

[a] Concentrate samples will be 2 - 5 gallons, containing equivalent solids to the feed sample.

3.0 PREPARATION OF TEST-SAMPLE CONTAINERS

3.1 Containers

The containers listed in Table 3 will be used for the test samples. Unless otherwise specified the caps will be lined with Teflon tape, 2 mils thick.

3.2 Cleaning Procedures

a. Narrow-mouth glass and amber glass test-sample bottles and caps

Wash with strong acid (50% H_2SO_4 and 50% HNO_3) and rinse several times with tap water and deionized water. Heat the bottles for thirty minutes at 400°C in a glass annealing oven then cool to room temperature and cap.

b. Cubitainers and caps

Rinse cubitainers and caps several times with deionized water, drain at room temperature, and cap.

c. Plastic bottles and caps

Wash with acid (5 mℓ of redistilled HNO_3 per liter of deionized water) and rinse several times with deionized water. Cap after draining at room temperature.

d. Glass vials and Teflon-lined septa

The glass vials are prepared as in 3.2 (a). Rinse the Teflon-lined septa several times with deionized water and dry at room temperature. Cap vials after cooling.

Table 13. DESCRIPTION OF TEST-SAMPLE CONTAINERS

Bottles	Supplier and Catalog Number	
480 mℓ, amber glass	A. H. Thomas	1702-N43
480 mℓ, glass	A. H. Thomas	1702-F70
480 mℓ, polyethylene	A. H. Thomas, high density polyethylene, Nalge 2002 series	1702-K63
5 gal, 1 gal cubitainers	Cole-Parmer Instrument Co.	6100-40, 6100-30
1 gal, amber glass	Fisher Scientific Co. ring jugs	2-884-5BB
40 mℓ, vials and septa	Pierce, Inc.	13075 12722

e. Teflon sheets for lining caps

Wash with acid (5 mℓ of redistilled HNO_3 per liter of deionized water) and rinse with deionized water.

4.0 PREPARATION OF HYPERFILTRATION APPARATUS AND FEED TANK

The tanks will be scrubbed, washed with a detergent, rinsed several times with plant water and drained. The process fluid will be transferred through a polypropylene filter to the feed tank using an existing industrial tube using a stainless steel pump. The process fluid will cool to room temperature in the feed tank until operation commences with the hyperfiltration apparatus. The feed tank will be kept covered to minimize escape of volatiles.

The hyperfiltration unit will be cleaned of residual material using a sequence of washes. A detergent operation followed by a base wash is expected to remove most greases, waxes, and organic materials. The unit will be rinsed with plant water. The unit will be operated with plant water to indicate whether materials are evolved within the plumbing or feed system. A sample (CTHF-2) will be analyzed for comparison with the plant water sample.

5.0 SAMPLING PROCEDURE

5.1 Collection Samples

A sample of the plant water will be obtained from the tap providing water for the scour operation (CTHF-1).

The feed samples will be collected during the transfer of the test fluids from the feed tank to the hyperfiltration apparatus (CTHF-3, CTHF-7, CTHF-10, CTHF-14).

The total permeate from each hyperfilter will be collected in a stainless steel container (prepared as described in 3.2 (c). The test samples will be taken from this collection container by draining or siphoning. The permeate samples are a composite of the permeate from each hyperfilter (CTHF-4, CTHF-5, CTHF-8, CTHF-11, CTHF-12, CTHF-15).

The concentrate samples will be obtained by draining the hyperfiltration apparatus at the completion of each of the four experiments (CTHF-6, CTHF-9, CTHF-13, CTHF-16).

5.2 Test Samples

Each collection sample will be divided into the specified test samples.

a. Bioassay test (B.1, B.2, B.3), nonvolatile solutes and pesticide (C.2 and C.6) and metals (C.3)

Remove sample container cap, fill completely by draining or siphoning from the collection container, cap immediately and cool to 4°C as rapidly as possible. No preservative is added.

b. Volatile solutes (C.1)

Collect two 40 mℓ samples. Slowly fill each vial to overflowing. Carefully set the vial on a level surface. Place the septum (Teflon side down) on the convex sample miniscus. Seal the sample with the screw cap. To insure the sample has been properly sealed, invert the sample and lightly tap the lid on a solid surface. The absence of entrapped air bubbles indicates a proper seal. If air bubbles are present, open the bottle, add additional sample, and reseal. Cool to 4°C as rapidly as possible. No preservative will be added.

c. Cyanide (C.4)

Collect sample as described in 5.2 (a). A preservative is required at the time of collection. Add 1.0 mℓ of 10 N NaOH, to obtain pH ≈ 12. Oxidizing agents such as chlorine decompose most cyanides. Test a drop of the sample at the time of collecting using KI-starch paper; a blue color indicates a need for chlorine treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper. Then add 0.3 g of ascorbic acid.

d. Phenols (C.5)

Collect sample as described in 5.2 (a). A preservative is required at the time of collection. Acidify the sample to pH = 4 by addition of phosphoric acid. Determine pH with pH paper. Note volume of acid added and its concentration on the sample tag.

5.3 Labels

Labels are to be waterproof and information written with India ink. Each label will indicate:

Collection Sample Number ______________

Test Sample Designation ______________

Sampler ______________

Date ______________

Time ______________

Preservatives ______________

6.0 SHIPPING PROCEDURES

All samples will be refrigerated upon collection. Cardboard cartons, suitably insulated, will be charged with water ice or dry ice and the samples placed therein. Care will be taken to insure the non-freezing of samples if dry ice is used. A refrigeration life of forty hours will be used as a design criterion.

Notice will be given in advance of testing to the recipients of test fluids and approximate scheduling. A definite notice including bill of lading number and anticipated flight schedule will be relayed upon shipment.

Shipments will be by air freight. Parcels will be marked "Contents under Refrigeration," "Perishable," "Handle with Care - Fragile," and "Contains Dry Ice" or "Contains Ice" as appropriate.

7.0 DEFINITIONS

PEA - poly(ether amide) hyperfilter

CA - cellulose acetate hyperfilter

DM - ZrO-PAA dynamic membrane hyperfilter

Collection Sample - designated samples in Table 1, e.g., CTHF-3

Test Sample - Sample of collection sample sent for a specific test. Designations are listed in Table 2. Example: Test sample CTHF-3, C.1 is the sample of collection sample CTHF-3 bottled in two 45 mℓ vials for volatile solids analyses.